BROOK'S CLINICAL PEDIATRIC ENDOCRINOLOGY

7TH EDITION

Edited by
Mehul T. Dattani
Charles G.D. Brook

WILEY

Brook's Clinical Pediatric Endocrinology

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Seventh Edition

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Preface

It is nearly 40 years since I wrote the preface to the first edition of this book. There have not been fundamental changes in the clinical practice of pediatric endocrinology during those years but the era of molecular biology has changed completely the understanding of the causation of many of the disorders seen and described in this edition of the book. In years to come it will impinge on clinical practice.

Major changes have occurred in the access to information through the internet and also in the ways that a book is now assembled, prepared and printed in different parts of the world. The ready access to original literature seemed likely to make text books like this redundant but the plethora and complexity of the information available makes even more relevant the authoritative digestion of data and their presentation in a clinically useful format. This has always been the aim of the book.

One loss with the internet is the close personal relationships which used to exist between editors, authors and their publisher; so many people are now involved in the actual production of a book that it is no longer possible to identify exactly who does what. Nevertheless I thank our authors and all at Wiley for their endeavours.

Although I have claimed the right to be the sole author of the preface for this edition of *Clinical Pediatric Endocrinology* (which will be the last in which I shall be involved), it will be clear that the brains behind the book are those of my long-term colleague, now mentor and friend, Mehul T. Dattani. His time in many roles in what was my department and is now his spans 30 years. I and many others respect and admire his achievements and this edition would never have seen the light of day without him. My contribution has been trying to make the book readable, which is not always an easy task.

The number of practitioners of clinical pediatric endocrinology worldwide has increased by at least two orders of magnitude since 1981 and so no longer is this edition dedicated to just the European Society for Paediatric Endocrinology and the Lawson Wilkins Pediatric Endocrine Society but to all who strive to advance our field. Lastly, I should acknowledge with love and gratitude the way my wife Catherine has put up with this cuckoo in our nest for so many years.

Charles G. D. Brook
Hadspen Farm, Somerset, UK

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I would also like to thank my children Seyan Dattani and Arushi Dattani for their help in checking the proofs.

About the Companion Website

This book is accompanied by a companion website:

www.wiley.com/go/dattani/brookcpe7

The website includes:

MCQs

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Genetics and Genomics

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Introduction

The Human Genome Project was completed in 2003 but it is only now that we are truly in the genomic era. Next-generation sequencing (NGS), which allows genome-wide detection of variants, is transforming on an unprecedented scale our understanding of pediatric and endocrine diseases by identifying mutations that are pathogenic or confer disease risk: new genes that cause human disease are being identified at the rate of 3 per week. We all differ in our DNA sequence and medical geneticists aim to understand the significance of this genetic diversity in health and disease, which has led to the age of genomic medicine.

Understanding genetic diversity is essential to understanding the biology of diseases of various kinds, from simple *Mendelian or monogenic disorders* to more complex *multifactorial disease*, and how we respond to treatment at both population and individual levels. We have the capacity to study the human genome as an entity rather than one gene at a time and medical and clinical genetics has become part of the broader field of genomic or *precision medicine*, which seeks to apply a large-scale analysis of the human genome to provide an individual and knowledge-based approach to medical care.

Many web resources and web-based tools have been developed to help the clinicians navigate and interpret the tremendous amount of genomic data that are being generated (Table 1.1).

The term '-omics' aims at the collective characterization and quantification of pools of biological molecules that translate into the structure, function and dynamics of an organism. Genomics can be divided into *comparative genomics*, the study of the relationship of genome structure and function across different biological species or strains; *functional genomics*, which describes gene and protein functions and interactions; *metagenomics*, the study of genetic material recovered directly from environmental samples; and *epigenomics*, which is the study of the complete set of epigenetic modifications on the genetic material of a cell, known as the epigenome.

Basic Concepts in Human Genetics and Genomics

Genes and Chromosomes

Genetic information is stored in DNA in the chromosomes within the cell nucleus. DNA is a polymeric nucleic acid macromolecule composed of a five-carbon sugar (deoxyribose), a nitrogen-containing base and a phosphate group. The bases are of two types, purines and pyrimidines. In DNA, there are two purine bases, adenine (A) and guanine (G), and two pyrimidine bases, thymine (T) and cytosine (C). DNA is organized in a helical structure in which two polynucleotide chains run in opposite directions, held together by hydrogen bonds between pairs of bases, A of one chain pairing with T of the other and G with C. In the coding sequences of a gene, each set of three bases constitutes a *codon* that encodes for a particular amino acid. *Genome* refers to the totality of genetic information carried by a cell or an organism, whereas *genotype* is the genetic constitution of an individual cell or organism. With the exception of cells that develop into gametes (the germline), all cells that contribute to the body are termed somatic cells.

The human genome contained in the nucleus of the somatic cells consists of 46 chromosomes arranged in 23 pairs, 22 of which are common in both males and females and are termed autosomes, and the remaining pair being the sex chromosomes, two X chromosomes in females and an X and a Y chromosome in males. *Homologous chromosomes* refer to members of a pair of chromosomes which carry the same genes in a similar organization.

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Companion website: www.wiley.com/go/dattani/brookcpe7

Table 1.1 Commonly used databases in human genetic and genomic analysis.

Site	Content	URL
National Center for Biotechnology Information	A portal that provides access to a wealth of biomedical and genomic information. Includes PubMed, OMIM, dbSNP, Clinvar, expression data sets. Suite of tools for data and sequence analysis (e.g. BLAST)	http://www.ncbi.nlm.nih.gov
Mendelian Inheritance in Man (MIM)	A comprehensive database of human genes and genetic disorders	http://www.ncbi.nlm.nih.gov/omim
ClinGen	Authoritative central resource that defines the clinical relevance of genes and variants for use in precision medicine and research	https://www.clinicalgenome.org
Ensembl	Genome browser for vertebrate genomes that supports research in comparative genomics, evolution, sequence variation and transcriptional regulation. Annotates genes, computes multiple alignments, predicts regulatory function and collects disease data	http://www.ensembl.org
University California, Santa Cruz (UCSC), genome browser	Genome browser offering access to genome sequence data from vertebrate and invertebrate species and major model organisms. Integrated with a large collection of analysis tools	https://genome.ucsc.edu
GeneCards	Provides comprehensive information on all human genes. It integrates gene data from ~125 web sources, including genomic, transcriptomic, proteomic, genetic, clinical and functional information	http://www.genecards.org
Human Gene Mutation Database (HGMD)	Collates published gene lesions responsible for human inherited disease	www.hgmd.cf.ac.uk/ac
Mouse Genome Informatics at the Jackson Laboratories	International database resource for the laboratory mouse, providing integrated genetic, genomic, and biological data to facilitate the study of human health and disease	http://www.informatics.jax.org
DECIPHER database	Collects clinical information about rare genomic variants and displays this information on the human genome map	https://decipher.sanger.ac.uk
Database of Genomic Variants (DGV)	A curated catalogue of human genomic structural variation	http://dgv.tcag.ca/dgv/app/home
Exome Aggregation Consortium (ExAC) browser	Exome data set >60,000 unrelated individuals. Provides both a reference set of allele frequencies and constraint metrics giving information on whether a gene is tolerant or intolerant to variation	http://exac.broadinstitute.org
ClinVar	Aggregates information about genomic variation and its relationship to human health.	http://www.ncbi.nlm.nih.gov/clinvar
Sequence Variant Nomenclature	Provides guidelines for sequence variation nomenclature	http://varnomen.hgvs.org
dbSNP	Genetic variation within and across different species. Not limited to SNPs, it contains a range of molecular variation	http://www.ncbi.nlm.nih.gov/SNP
F-SNP	Provides integrated information about the functional effects of SNPs obtained from 16 bioinformatics tools and databases. Helps identify and focus on SNPs with potential pathological effect to human health	http://compbio.cs.queensu.ca/F-SNP
Biological General Repository for Interaction Datasets (BioGRID)	Database of protein–protein interactions, genetic interactions, chemical interactions, and post-translational modifications	http://thebiogrid.org

Table 1.1 (Continued)

Site	Content	URL
PhenomicDB	A multi-organism phenotype–genotype database including human, mouse, fruit fly, *C. elegans*, and other model organisms	http://www.phenomicdb.de
Phencode	Connects human phenotype and clinical data in various locus-specific mutation databases with data on genome sequences, evolutionary history and function in the UCSC Genome Browser	http://phencode.bx.psu.edu
Human Epigenome Atlas	Includes human reference epigenomes and the results of their integrative and comparative analyses. Provides details of locus-specific epigenomic states like histone marks and DNA methylation across tissues and cell types, developmental stages, physiological conditions, genotypes and disease states	http://www.genboree.org/epigenomeatlas
Encyclopedia of DNA Elements (ENCODE)	Catalogue of functional elements in the human genome, including elements that act at the protein and RNA levels and regulatory elements that control cells and circumstances in which a gene is active	https://www.encodeproject.org
Genomics England 100,000 Genomes Project	The project will sequence 100,000 genomes from around 70,000 people. Participants are National Health Service (UK) patients with a rare disease, plus their families, and patients with cancer	www.genomicsengland.co.uk/the-100000-genomes-project

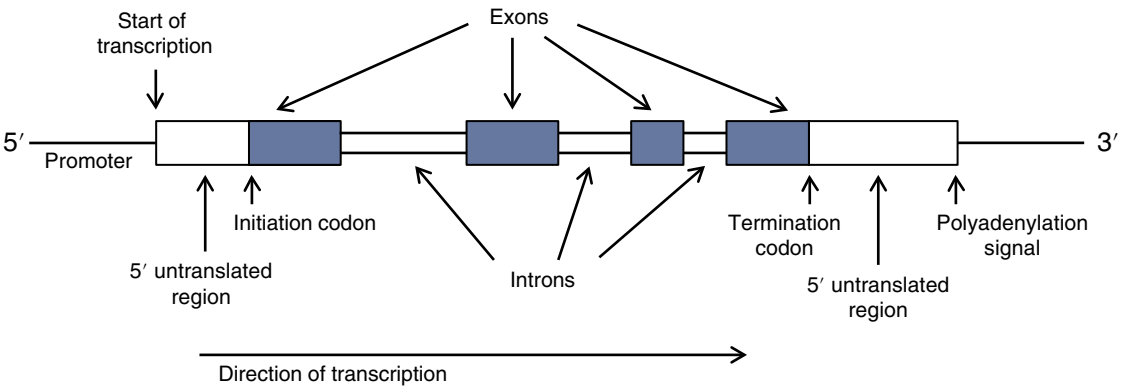

Figure 1.1 Example of a typical mammalian gene structure. A typical gene has regulatory regions preceding the coding exons interspersed by non-coding introns. The individual labelled features are discussed in detail in the text.

A *gene* is a sequence of DNA in the genome required for the expression of a functional product, including a polypeptide or RNA molecule (Figure 1.1). The majority of human genes are organized as coding regions called *exons* interrupted by one or more non-coding regions termed *introns*. Introns are initially transcribed into RNA in the nucleus but are not present in mature mRNA, which also has flanking 5′ and 3′ *untranslated regions* (UTRs). The latter contains a signal for the addition of adenosine residues (the polyA tail) to the end of the mature mRNA.

Other sequences within the 3′ UTR are important for translation efficiency, localization and stability, whereas the 5′ UTR is important in the regulation of RNA translation. It is important when discussing genes to define what is meant by the terms *trans* and *cis*. *trans*-Acting usually means 'acting from a different molecule', whereas *cis*-acting means 'acting from the same molecule'. In genetics and genomics, *cis*-acting elements refer to DNA sequences in the vicinity of a gene that are required for gene expression; trans-acting factors, either proteins or some classes of RNA molecules, bind to the *cis*-acting sequences to control gene expression.

Many genes produce not just one but multiple proteins, which is achieved either by *alternative splicing* of the coding segments of genes or by numerous types of

biochemical modifications of the resulting proteins so that the 19,000 genes in the human genome are estimated to generate over a million different proteins. The other point to remember is that individual proteins rarely work by themselves. The cell is composed of modular supramolecular complexes and each complex performs an independent, discrete biological function that could not be achieved by the independent components of the complex. The transfer of information from the DNA strand to the protein is mediated by RNA, which directs the synthesis and sequence of polypeptides.

Genetic information is stored in genes in the form of a genetic code in which the sequence of adjacent bases determines the sequence of amino acids in the polypeptide. RNA is synthesized from DNA by *transcription* and the RNA carrying the coded information is termed *messenger RNA* (mRNA), which is transported from the nucleus to the cytoplasm where it is translated to synthesize the protein. This constitutes the central dogma of molecular biology.

Regulation of Gene Expression

Gene expression is the production of correct RNA, which is a complex process where the RNA must be expressed in the appropriate cell type in the correct amount and, in some cases, at a precise developmental time. Nucleic acid sequences flanking the coding sequences and in some cases within the coding sequences provide the molecular signals for gene transcription. A *promoter* region that contains sequences necessary for the initiation of transcription lies at the 5′ end of most genes. An *enhancer* is a short (50–1500 bp) region of DNA that can be bound by proteins (transcription factors) to increase the likelihood that transcription of a particular gene will occur. Enhancers are generally located up to 1 Mbp away from the gene and can be upstream or downstream of the gene it regulates. The orientation of an enhancer may even be inverted without having an effect on its function.

Genes that are necessary for complex and multiple developmental processes usually have a number of enhancers with overlapping functions. A good example is the *SOX9* locus: the developmental timing and tissue-specific transcriptional regulation of *SOX9* are highly complex and involve multiple elements located in flanking regions of at least 1 Mb upstream and 1.6 Mb downstream and these show strikingly different phenotypes when mutated. Upstream rearrangements are associated with campomelic dysplasia and fall within two clusters located about 400 kb apart. Large (>1 Mb) duplications 5′ to *SOX9* (i.e. downstream) are associated with brachydactyly anonychia (symmetric brachydactyly of the hands/feet, hyponychia or anonychia). Pierre Robin sequence (micrognathia, cleft palate and macroglossia) is caused by either a deletion located 1.38 Mb upstream or a deletion located 1.56 Mb downstream of *SOX9*.

Another regulatory element, termed *RevSex*, is located 600 kb upstream of *SOX9* gene. Three copies of *RevSex* are associated with testicular or ovotesticular disorders of sex development (DSD), whereas deletions (one copy) of *RevSex* are associated with 46,XY gonadal dysgenesis.

RNA Editing

RNA editing is a molecular process through which cells can make discrete changes to specific nucleotide sequences within a RNA molecule. RNA editing includes nucleotide additions and insertions as well as nucleobase modifications such as cytidine (C) to uridine (U) and adenosine (A) to inosine (I), which is termed deamination. RNA editing in mRNAs alters the amino acid sequence of the encoded protein so that it differs from that predicted by the genomic DNA sequence.

Various post-translational modifications after protein biosynthesis can extend the chemical repertoire of the 20 amino acids by introducing new functional groups such as phosphate, acetate, amide groups or methyl groups. This can occur on the amino acid side chains or at the protein's carboxy (C-) or amino (N-) termini. The most common post-translational modifications in descending order are phosphorylation, acetylation, N-linked glycosylation, palmitoylation and O-linked glycosylation.

Classes of RNA Molecules and their Functions

In recent years there has been a substantial shift in understanding the importance of different classes of RNA molecules in biological processes. Ninety percent of the human genome is transcribed, but many of the resulting transcripts and the factors regulating their transcription remain uncharacterized. The vast majority of the transcribed genome comprises diverse classes of *non-coding RNAs* (ncRNAs) that may play key roles in different biochemical and cellular processes with profound implications for human health and disease. The cellular repertoire of ncRNAs consists of small housekeeping RNAs, such as *ribosomal RNAs* (rRNAs) and *transfer RNAs*, *microRNAs*, and *long ncRNAs* (lncRNAs). Assigning molecular, cellular, and physiological functions to well-annotated ncRNAs is the current challenge in this field. ncRNAs are emerging as key players in pediatric and endocrine diseases.

MicroRNAs, Small Interfering RNAs, and Piwi-interacting RNAs

Small RNAs usually refers to any class of non-coding RNA of 19–32 nt (Table 1.2). The role of small RNAs is continuing to be explored but their main function is *gene silencing* through RNA-mediated mechanisms. *RNA silencing* is an umbrella term for all small RNA-mediated inhibition of transcription, translation and deactivation of transposable elements. RNA silencing is widely regarded as a master controller of gene regulation but small RNAs have important roles in an increasing variety of eukaryotic biological processes. For example, small RNAs may play a role in transgenerational inheritance and epigenetic memory.

In eukaryotes, there are three major classes of small RNAs involved in post-transcriptional regulation: microRNAs (miRNAs), small interfering RNAs (siRNAs) and Piwi-interacting RNAs (piRNA). All find target RNAs by base pairing between complementary sequences, causing target RNA degradation and/or translational repression. Each type has its own preferred class of RNA targets, reflecting their biological functions. miRNAs and siRNAs bind to proteins in the Argonaute subfamily, whereas piRNAs bind to the Piwi subfamily of proteins.

MicroRNAs (miRNAs) are an abundant class of small evolutionarily conserved regulatory RNAs about 19–22 nucleotides (nt) in length. They are thought to play fundamental roles in most biological processes including disease. Over 1500 miRNAs have been identified that regulate the expression of up to 60% of mammalian genes. The canonical miRNA pathway starts with the transcription of miRNA genes by RNA polymerase II, which results in the production of the primary miRNA (pri-miRNA). The pri-miRNA transcript is cleaved by a protein complex consisting of Drosha/DGCR8 to generate an ~80 base-pair precursor miRNA (pre-miRNA) with a characteristic hairpin secondary structure vital for enabling export from the nucleus.

Dicer, an RNase III/helicase multi-domain enzyme, processes the pre-miRNA into a ~22 bp miRNA. The gene encoding DICER is termed *DICER1*. *DICER1* syndrome is an inherited disorder that increases the risk of a variety of cancerous and benign tumours, including pleuropulmonary blastoma, cystic nephroma, multinodular goiter and Sertoli–Leydig cell tumours of the ovary, which typically develop in affected women in their teens or twenties. Some Sertoli–Leydig cell tumours release testosterone resulting in virilization.

Incorporated into one member of the Argonaute (Ago) protein family is the RNA-induced silencing complex (RISC), a mature miRNA that binds typically to the 3′-UTR of the mRNA and inhibits its translation via various mechanisms including mRNA degradation. The key determinant of target recognition is a short sequence complementarity between the miRNA seed sequence (the second–eighth nucleotides of the miRNA) and the target mRNA. The maturation and function of miRNAs are highly dependent on the coordinated action of several RNA-binding proteins.

The miRNA genes are mainly clustered in the genome and are transcribed as *polycistronic* primary transcripts. Forty percent of miRNA genes lie in the introns of protein and non-protein coding genes. These are usually,

Table 1.2 Characteristics of the three major classes of small RNAs involved in post-transcriptional regulation.

Properties	miRNA	piRNA	siRNA
Size (nt)	20–24 (usually 22)	26–31	20–25
Origin	Endogenous and ubiquitous	Endogenous to germ cell lineages	Exogenous or endogenous
Evolutionary conservation	Eukaryotes	Vertebrates and invertebrates	Eukaryotes
Precursor	Single-stranded RNA	Single-stranded RNA	Double-stranded RNA
Biogenesis	Dicer dependent	Dicer independent	Dicer dependent
Base-pair match to target	Imperfect	Perfect	Perfect
Distribution	Cytoplasmic and nuclear	Cytoplasmic and nuclear	Cytoplasmic
Ago dependence	Ago subfamily	Piwi subfamily	Ago subfamily
Target nucleic acid	3′UTR, 5′-UTR, promoters, coding regions, pseudogenes	Transposons	mRNA, promoters
Main functions	Translation inhibition, mRNA degradation, transcriptional and post-transcriptional silencing	Transposon silencing, transcriptional and post-transcriptional silencing	mRNA degradation, transcriptional and post-transcriptional silencing

though not exclusively, found in a sense orientation and thus are regulated together with their host genes. Pre-miRNAs that are spliced directly out of introns are termed *Mirtrons*. Approximately 16% of pre-miRNAs are modified by nuclear RNA editing, leading to changes in biological function. This is mainly mediated by adenosine deaminases acting on RNA to catalyse adenosine to inosine (A to I) transitions. RNA editing can result in disruption of nuclear processing as well as alter downstream processes including cytoplasmic miRNA processing and target specificity.

Gene regulation by miRNAs is of key importance in many fundamental biological processes such as cellular differentiation, proliferation, migration and apoptosis. In disease, some circulating blood miRNA levels are proportional to the degree of severity of the pathology, such as drug-induced liver injury, cardiovascular infection, cancer, Alzheimer's disease, inflammation and metabolic diseases (obesity). Altered expression of miRNAs in diabetes causes malfunction of insulin release and insulin resistance. The use of miRNAs as biomarkers for type 1 diabetes (T1D) risk is attractive as these markers could be used to identify individuals at risk for developing T1D before symptoms appear. Twelve miRNAs were found to be more concentrated in sera from children and adolescents with newly diagnosed T1D compared with sera from age-matched controls. Among them, miR-25 was associated with improved glycaemic control and better residual β-cell function, suggesting that this miRNA could be used during early and intensive management of newly diagnosed diabetes to improve blood glucose control and reduce microvascular complications.

piRNA is the largest class of small non-coding RNA molecules expressed in animal cells. piRNAs are generated from various portions of long single-stranded precursor RNAs transcribed from genomic loci termed piRNA clusters, which are often >100 kb in size. They are distinguished from miRNA by their size (26–31 nt), lack of sequence conservation and increased sequence complexity. The majority are antisense to transposon sequences, indicating that transposons are the piRNA target. piRNAs direct the Piwi proteins to their transposon targets for gene silencing. The piRNA-mediated repression of transposons is best characterized in the germline. piRNAs are necessary for spermatogenesis in humans.

siRNAs are derived from long double-stranded precursor RNAs (dsRNAs). Endogenously formed dsRNAs are exported to the cytoplasm where they are cleaved into 20–25-nt duplexes by Dicer. One strand of these fragments, usually the antisense strand, is incorporated into multiprotein RISCs composed of one of a family of Argonaute proteins together with auxiliary proteins that extend or modify the function. In contrast to miRNAs, siRNAs have a sequence fully complementary to their target mRNA and usually have a single target mRNA. Depending on the source of dsRNA precursor, siRNAs can be further divided into exogenous and endogenous siRNAs (exo- and endo-siRNAs, respectively).

LncRNAs

LncRNAs, non-protein-coding RNA transcripts longer than 200 nucleotides (nt), are emerging as key regulators of diverse cellular processes. The definition of lncRNAs continues to evolve. The first reported example of a long non-coding RNA (lncRNA) was the *H19* transcript, which lacked large open reading frames and was not translated into protein. Later work revealed the existence of thousands of lncRNAs in the human genome. The expression of lncRNAs is usually low but they are transcribed in a highly regulated manner, either from their own promotor sequence or as a by-product of other transcriptional processes. Although some lncRNAs are located within intergenic sequences, the majority are transcribed as complex, interlaced networks of overlapping sense and antisense transcripts that often include protein-coding genes. They are generally, but not exclusively, spliced, 5′-capped and 3′-polyadenylated, and transcribed by RNA polymerase II.

Approximately one-third to one-half of lncRNAs overlap protein-coding genes. Genic lncRNAs can be further divided into those that overlap protein-coding loci in the sense or antisense direction and those that overlap exonic or intronic regions of the protein-coding gene. A universal classification does not exist. The 200-nt cut-off to define their size is arbitrary and does not represent a biological distinction. A lncRNA may code for a polypeptide but it must have coding-independent functions, as shown for the steroid receptor RNA activator (SRA), a well-characterized bifunctional lncRNA involved in the nuclear receptor-mediated regulation of gene expression. The SRA1 gene expresses both SRA RNA and the SRA protein (SRAP). This gene is involved in the regulation of many NR and non-NR activities, including metabolism, adipogenesis and chromatin organization. The encoded protein, SRAP, acts as a transcriptional repressor by binding to the non-coding RNA. SRA coactivates a range of nuclear receptors including ERα and ERβ in a ligand-dependent manner by direct interaction with other co-regulatory proteins. lncRNAs are becoming increasingly important in oncology. The dysregulation of lncRNAs expression is highly specific to the cancer type as compared to the protein-coding genes. lncRNAs are being identified as drivers of cancer with their potential functions being predicted. This is providing a framework for the development of new cancer diagnostics, stratification and precision treatments.

Other Small ncRNA Classes

Small nucleolar RNAs (snoRNAs) are a class of small RNA molecules that primarily guide chemical modifications of other RNAs, mainly ribosomal RNAs, transfer RNAs and small nuclear RNAs. There are two main classes, the C/D box snoRNAs, which are associated with methylation, and the H/ACA box snoRNAs, which are associated with pseudouridylation.

Y non-coding RNAs were initially found in the cytoplasm of mammalian cells. There are four non-coding Y RNAs in humans: hY1, hY3, hY4 and hY5 RNA. Y RNA fragments are not involved in the miRNA pathway but they are essential factors for the initiation step of chromosomal DNA replication in human cell nuclei. The mechanism is poorly understood but is thought to be mediated by interactions with chromatin and transcription initiation proteins.

Small nuclear ribonucleic acid (snRNA) are found within the splicing speckles and Cajal bodies of the cell nucleus in eukaryotic cells. The length of an average snRNA is ~150 nucleotides. Their primary function is the processing of pre-messenger RNA in the nucleus. They also aid the regulation of transcription factors (7SK RNA) or RNA polymerase II (B2 RNA) and telomere maintenance.

Circular RNAs (CircRNAs) are a family of naturally occurring endogenous ncRNAs with widespread distribution and diverse functions. These ~100 nucleotides long, single-stranded RNA molecules form a circle through covalent binding. CircRNAs mainly arise from the exons of protein-coding genes but they can also be derived from introns, untranslated regions, intergenic loci and antisense sequences of known transcripts. CircRNAs are common in the eukaryotic transcriptome and abundant in exosomes. CircRNAs show a high sequence conservation with specific expression in various tissues during different developmental stages. Some circRNAs can interact with miRNAs and can function as miRNA sponges in mammalian cells. CircRNAs are becoming increasingly important in medicine by serving as biomarkers for non-invasive diagnosis of atherosclerosis, neurodegenerative diseases and cancers.

Gene Mutations and Inheritance

Any permanent heritable change in the sequence of genomic DNA is termed a *mutation*.

Classes of Gene Mutations

Fifty percent of all disease-causing mutations are *missense,* which are caused by a single nucleotide substitution (*point mutation*) in the DNA coding sequence of a gene that results in the replacement of one amino acid by another in the final protein product. Nucleotide changes that involve the substitution of one purine for the other (A for G or G for A) or one pyrimidine for the other (C for T or T for C) are termed *transitions.* The replacement of a purine for a pyrimidine (or vice versa) is a *transversion*. Missense mutations are often referred to as *nonsynonymous* mutations, whereas point mutations that do not alter the amino acid in the final protein product are referred to as *synonymous* mutations. Although the latter were largely ignored since they were considered to have no functional consequences, there is a growing realization that they can be associated with disease by affecting the stability of the mRNA, mRNA folding, translation fidelity and miRNA–mRNA interaction or by creating novel RNA splice sites. More than 50 human diseases are associated with synonymous mutations including Crohn's disease, Treacher Collins syndrome and Crouzon syndrome.

A point mutation in a DNA coding sequence that results in the replacement of an amino acid codon to one of the three termination codons is termed a *nonsense mutation*. Depending on its position, the resulting transcript is predicted to be recognized by the nonsense-mediated decay surveillance complexes and degraded. If this does not occur, the resulting truncated protein is usually unstable and degraded. Ten percent of all disease-causing mutations are nonsense mutations, which may affect the processing of RNA. For introns to be excised from unprocessed RNA and the exons spliced together to form a mature mRNA requires a specific nucleotide sequence located at the exon–intron (5′ donor site) or the intron–exon (3′ acceptor site) junctions. Mutations that affect these required bases at either the splice donor or acceptor site interfere with (and in some cases abolish) normal RNA splicing at that site. A second class of splicing mutations involves intron base substitutions that do not affect the donor or acceptor site sequences themselves. This class of mutations creates alternative donor or acceptor sites that compete with the normal sites during RNA processing. Thus, at least a proportion of the mature mRNA in such cases may contain improperly spliced intron sequences.

Mutations can also be caused by the insertion, inversion, translocation or deletion of DNA sequences. This may involve only a single base pair or up to several million base pairs. *Frameshift mutations* occur when small deletions or insertions occur within coding sequences and involve a number of bases that are not a multiple of 3. These generate a different sequence of codons from the point of the insertion or deletion and usually generate a downstream termination codon.

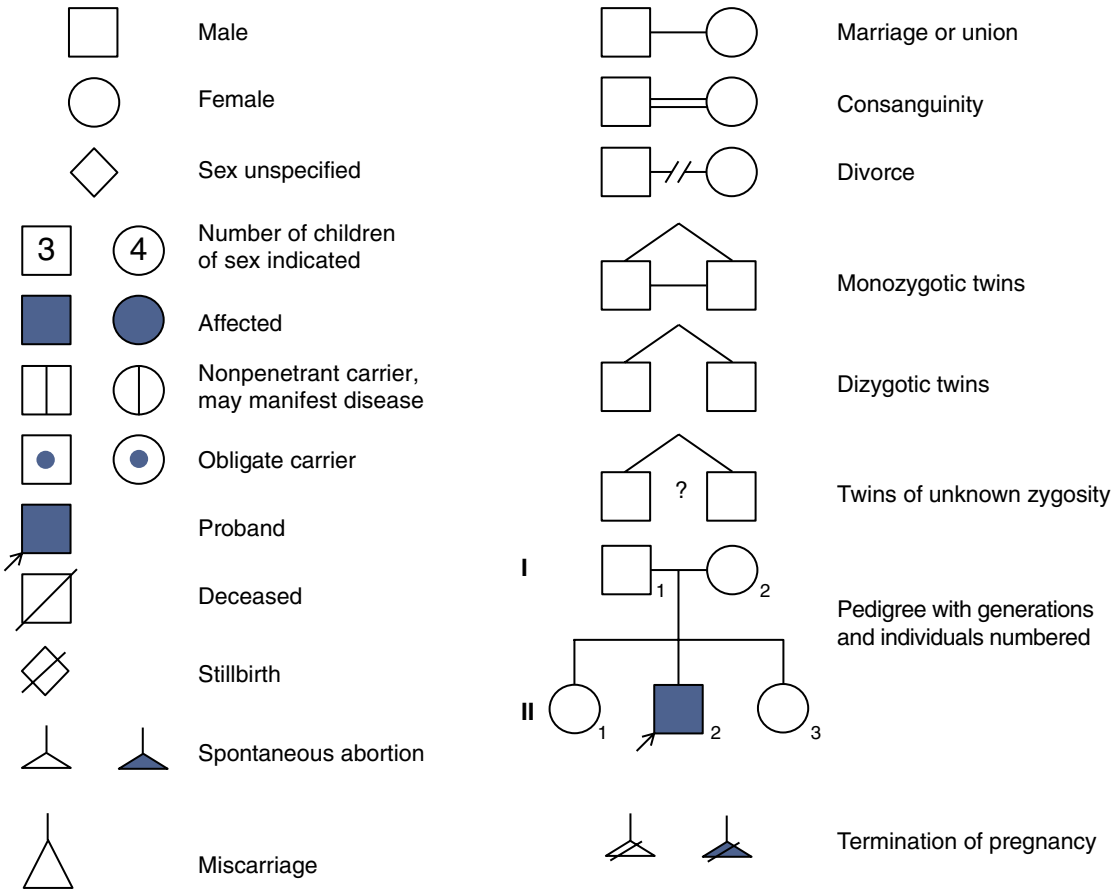

Figure 1.2 Symbols commonly used for creating pedigree charts.

Another form of mutation is *gene conversion*, a process by which one DNA sequence replaces a *homologous* sequence so that the sequences become identical after the conversion event. Gene conversion can be allelic, meaning that one allele of the same gene replaces another allele, or ectopic, meaning that one *paralogous* DNA sequence is converted to another. Gene congential plays an important role in congenital adrenal hypoplasia due to mutations involving the *CYP21A2* gene, which is located on chromosome 6p21.3, 30 kb from the *CYP21A1P* pseudogene. Both *CYP21A2* and *CYP21A1P* show sequence identity of 98% between exons and 96% between introns. The high sequence identity and close proximity of *CYP21A2* and *CYP21A1P* can generate gene conversion that results in the transfer of deleterious mutations from the pseudogene to *CYP21A2*. It is estimated that 25% of all disease-causing mutations are due to deletions or insertions.

Patient and Family History

A detailed patient and family history and thorough clinical and biochemical investigation are essential to understand if the disorder follows one of five basic modes of inheritance for single-gene diseases or if the inheritance pattern follows a more complex inheritance with incomplete penetrance or variable expressivity. The family history should include the drawing of the pedigree where individuals are represented by symbols (usually circles for female and squares for male), solid to indicate someone affected by a trait and unfilled for unaffected. Figure 1.2 illustrates some of the conventions followed in constructing pedigrees. A detailed pedigree analysis can reveal the inheritance patterns in a family. Pedigree analysis is also useful for analysing populations with limited progeny data from multiple generations.

Mendelian Inheritance Patterns

The basic laws of inheritance are important to understand patterns of disease transmission. Single-gene or monogenic diseases are usually inherited in one of several patterns depending on the location of the gene in the genome or whether one or two copies of the gene are needed for normal biological activity. The five modes of inheritance for single-gene diseases are *autosomal*

Table 1.3 Types of inheritance and their associated family history.

Type of inheritance	Family history pattern
Autosomal dominant	Individuals carrying one mutated copy of the gene will be affected by the disease. Each affected person usually has one affected parent, although *de novo* mutations occur. Usually occurs in every generation of the family
Autosomal recessive	Individuals carrying two mutated copies of the gene will be affected. Parents are usually unaffected and each must carry a copy of the mutated gene (carriers). Usually not seen in every generation
Mitochondrial	Maternally inherited. Both males and females can be affected. Can appear in every generation of a family
X-linked dominant	Females are more frequently affected than males. Fathers cannot pass on X-linked traits to their sons
X-linked recessive	Males are more frequently affected than females. Affected males are often seen in each generation. Both parents of an affected daughter must be carriers. Only the mother must be a carrier of an affected son

dominant, *autosomal recessive*, *X-linked dominant*, *X-linked recessive* and *mitochondrial inheritance* (Table 1.3). Single-gene disorders affect 2% of the population sometime during a lifetime.

Single-gene disorders are dominant or recessive. A dominant phenotype is expressed in both homozygotes and heterozygotes for a mutant allele, whereas a recessive phenotype is expressed only in homozygotes for the mutant allele. Most dominant disorders are rare and usually seen in the heterozygous state. Thus approximately one-half of offsprings will inherit a dominant trait. Homozygotes for dominant traits have usually a more severe phenotype or fail to survive. Many autosomal dominant mutations result in *haploinsufficiency*, which occurs when the single functional copy of the gene does not produce enough gene product to produce a wild-type phenotype.

A *dominant negative mutation* dominantly affects the phenotype by means of a defective protein or RNA molecule that interferes with the function of the normal gene product in the same cell. Most recessive disorders are due to mutations that result in the reduction or elimination of the function of the gene product and are termed *loss-of-function* mutations. Examples include 5-alpha reductase deficiency, due to autosomal recessive mutations in the *SRD5A2* gene, and congenital adrenal hyperplasia, due to homozygous mutations in the *CYP21A2*, *CYP11B1* or *CYP11A1* genes.

The majority of loci on the X chromosome show *X-linked inheritance* because they participate in meiotic recombination only during female gametogenesis, when there are two X chromosomes; they cannot recombine with the Y during male gametogenesis. Males have a single X and are therefore *hemizygous* with respect to X-linked genes. 46,XY males are never heterozygous for alleles at X-linked loci, whereas females can be heterozygous or homozygous at X-linked loci. To compensate for the double complement of X-linked genes in females, alleles for most X-linked genes are expressed from only one of the two X chromosomes in any given cell.

A *sex-limited trait* is a phenotype expressed in only one sex, although the gene that determines the trait is carried by both sexes and therefore autosomal. This is an on or off phenomenon. Sex-limited genes cause the two sexes to show different phenotypes, despite having the same genotype. They are responsible for sexual dimorphism, a phenotypic (directly observable) difference between males and females of the same species (e.g. lactation). This is not to be confused with *sex-linked traits*, which is the phenotypic expression of an allele present on the sex chromosome of the individual. A classic example is male-limited precocious puberty, an autosomal dominant disorder in which affected boys develop secondary sexual characteristics and undergo an adolescent growth spurt at about 4 years of age. In some families, the phenotype is due to mutations in the gene that encodes the receptor for luteinizing hormone (*LCGHR*). A *sex-influenced trait* refers when the expressivity of the phenotype is influenced by the sex, for example, body and facial hair.

For many phenotypes the mode of inheritance may depend on the gene involved. Non-syndromic disorders of testis determination, 46,XY complete or partial gonadal dysgenesis, can be inherited in a number of different ways that include sex-limited autosomal recessive (e.g. *DHH*), sex-limited autosomal dominant with variable expressivity and incomplete penetrance (e.g. *NR5A1*), Y-linked (*SRY*) or X-linked (hemizygous duplication of *NR0B1*).

Non-Mendelian Inheritance Patterns

Mitochondrial DNA (mtDNA) molecules, are present in tens to thousands of copies per cell. If a cell contains mitochondria that contain only a pure population of mutant mtDNA, it is termed *homoplasmy*. Alternatively, if the cell has mitochondria, some with and some without mutation, it is termed *heteroplasmy*. Disorders involving mtDNA mutations are characterized by maternal inheritance. Sperm mitochondria are eliminated from the embryo, so that mtDNA is always inherited from the mother. Thus, all the children of a female who is

homoplasmic for a mtDNA mutation will inherit the mutation, whereas none of the offspring of a male carrying the same mutation will do so. mtDNA mutations cause human diseases that often involve the central nervous and musculoskeletal systems. The proportions of normal and mutant mtDNA in the cells making up different tissues often result in incomplete penetrance of the phenotype, variable expression and *pleiotropy*.

Amplification of a repeat sequence is observed in disorders such as Huntington disease and fragile X syndrome. In the former, a simple trinucleotide repeat is located in the coding region and in a transcribed but untranslated region of the *FRM1* gene in the latter. *Trinucleotide repeat* disorders often show a *parent-of-origin* effect. Large expansions of the CAG repeat that cause juvenile Huntington disease are generally of paternal origin, whereas large expansions of the CGG repeat in fragile X syndrome are often of maternal origin.

Mosaicism is the presence of at least two cell lines that differ genetically but are derived from a single zygote in an individual or a tissue. Mosaicism can be categorized as somatic and/or germline. Mosaicism for numerical or structural abnormalities of chromosomes is clinically important and somatic mutations are recognized as a major contributor to many types of cancer. Somatic mosaicism refers to population of cells that carry a mutation in some tissues of the body but not in the gametes, whereas the cells carrying the mutation may be restricted to the gamete lineage in germline mosaicism. In some individuals both somatic lineages and the germline may be affected. 45,X/46,XY is a disorder of sex development associated with sex chromosome aneuploidy and mosaicism of the Y chromosome associated with highly variable clinical phenotypes, ranging from partial virilisation and ambiguous genitalia at birth to individuals with completely male or female gonads.

Common Disorders with Complex Inheritance Patterns

Many common disorders such as myocardial infarction, Alzheimer disease and diabetes do not follow Mendelian inheritance patterns seen in single gene disorders. They result from complex interactions between a number of genetic and environmental factors and follow a multifactorial or complex inheritance pattern.

Complex phenotypes can be divided into *qualitative* and *quantitative traits*. A qualitative trait is the presence or absence of the disorder, whereas a quantitative trait is a measurable physiological or biochemical aspect of the disorder such as the body mass index in obesity.

Familial aggregation of a common phenotype does not always mean that the cause must be genetic. Family members may develop a disorder by chance since, as well as genes, family members often share a common environment, diet, socio-economic status and culture. The familial aggregation of a disorder can be measured by comparing the frequency of the disorder in the relatives of an affected proband with its frequency in the general population. The more common a disorder in the general population, the more likely it is that the familial aggregation may be a coincidence.

Another approach to determine familial aggregation is case–control studies. Patients with the disorder are compared with carefully chosen control individuals who do not have the disorder. Often this is a spouse since they usually match the case in terms of age, ancestry (previously referred to as ethnicity) and environment. These types of studies are subject to errors that include ascertainment biases and failure to correctly match case–control subjects. Control individuals should differ from cases only in their disease status. All other factors should be matched. If they are not matched, a case–control study may find significant associations that are due to differences in, for example, ancestry rather than any relationship to the presence or absence of the disorder.

The genetic contribution to a complex disorder can be dissected by measuring allele sharing between affected and unaffected relatives. The concept is simple: when a genetic contribution is important in a disorder, the frequency of disease concordance increases as the degree of relatedness increases. Monozygotic twins (MZ) are the most extreme example as they have all their alleles in common. A first-degree relative shares ½ of alleles, a second-degree relative ¼, and a third-degree relative ⅛ and so on. One of the most common ways of separating the genetic contribution from the environment is to study MZ and dizygotic twins (DZ). DZ reared together allow the measurement of disease concordance in a similar environment, whereas MZ twins provide the opportunity to study genotypically identical individuals reared in similar or different environments. Greater disease concordance in MZ versus DZ twins is strong evidence for a genetic contribution to the disease.

Heritability was developed to quantify the role of genetic differences in determining variability of quantitative traits. It is the fraction of the total phenotypic variance of a quantitative trait caused by genes. Due to genetic differences between individuals, the higher the heritability, the greater the variability of the phenotype. Heritability measures the fraction of phenotype variability that can be attributed to genetic variation but it does not indicate the degree of genetic influence on the development of a trait of an individual.

Good examples of complex or multifactorial disorders include hypospadias and T1D. Hypospadias is one of the most common congenital disorders in males occurring in 1:200–1:300 male births. Anterior (glandular) and

middle (penile) forms comprise 70–80% and 15–20% of cases, respectively. Hypospadias shows familial clustering, with 7% of cases having affected first-, second- or third-degree relatives. The chance that a brother of an affected boy will also have hypospadias is 9–17%. Studies of family as well as twins of known zygosity have estimated the heritability of hypospadias to be 57–77%, meaning that 57–77% of the phenotypic variability can be attributed to genetic variability. Like many other common disorders, such as infertility, current data indicate that hypospadias might be monogenic in a small proportion of the families (e.g. the mutations in genes *NR5A1, AR, FGFR2* and *MAMLD1*) but that there is a multifactorial cause in the majority of cases.

T1D is a result of interplay between genetic predisposition, environmental factors and reprograming of the immune system. The destruction of pancreatic β-cells affects the level of insulin secretion leading to disease development. Destruction of pancreatic β-cells is mediated by an altered immune response due to genetic anomalies resulting in increase of pro-inflammatory cytokines and autoreactive T and B lymphocytes. Twin studies have estimated that 88% of phenotypic variance of T1D in Finland is due to genetic factors and the remaining due to unshared environmental factors. Genome-wide association studies have identified more than 50 variants associated with increased risk for T1D. The HLA class II region has the strongest impact on T1D risk. However, more than 40 non-HLA loci that impact upon the risk of developing T1D have been identified. Many of these genes are associated with immune function including *interleukin (IL)-2Ra*, *PTN22*, *IL-10*, *CCR5* and *IL-2*.

Uniparental Disomy

Uniparental disomy (UPD) occurs when a person receives two copies of a chromosome or of part of a chromosome from one parent and no copy from the other. UPD usually arises from the failure of the two members of a chromosome pair to separate properly into two daughter cells during meiosis in the parent's germline (nondisjunction). The resulting gametes contain either two copies of a chromosome (disomic) or no copy of that chromosome (nullisomic). This leads to a conception with either three copies of one chromosome (trisomy) or a single copy of a chromosome (monosomy).

If a second event occurs by either the loss of one of the extra chromosomes in a trisomy or the duplication of the single chromosome in a monosomy, the karyotypically normal cell may have a growth advantage compared to the aneuploid cells.

A postfertilization error can also lead to UPD, by either somatic recombination or gene conversion. Two types of UPD can be defined – uniparental heterodisomy (UPhD), where the two different alleles of the same parent are transmitted, and uniparental isodisomy (UPiD), where two identical copies of one allele of the contributing parent are present.

UPD may have clinical relevance for several reasons. For example, either *isodisomy* or *heterodisomy* can disrupt parent-specific genomic imprinting, resulting in imprinting disorders. Additionally, isodisomy leads to large blocks of homozygosity, which may lead to the uncovering of recessive genes. Uniparental inheritance of imprinted genes can result in phenotypic anomalies. Examples include Prader–Willi, Angelman and Silver–Russell syndromes. Prader–Willi syndrome, characterized by hypothalamic–pituitary abnormalities, is caused by deletion or inactivation of genes on the paternally inherited chromosome 15, while the maternal copy, which may be of normal sequence, is imprinted and therefore silenced. Angelman syndrome is a neurodevelopmental disorder caused by the loss of maternally inherited genes on chromosome 15 and paternal imprinting. Silver–Russell syndrome is a clinically heterogeneous disorder characterized by severe *in utero* growth restriction and poor postnatal growth, body asymmetry, irregular craniofacial features and several additional minor malformations. The etiology is complex and current evidence strongly implicates imprinted genes. Approximately half of all patients exhibit DNA hypomethylation at the *H19/IGF2* imprinted domain; around 10% have maternal UPD of chromosome 7.

Penetrance and Expressivity

Some disorders are not expressed in an individual, even though the individual carries the mutation causing the phenotype in other members of the family. This is termed *penetrance*, defined as the probability that a gene mutation will have a phenotypic expression. Penetrance is an all or nothing concept. If only a proportion of people carrying the genotype display the phenotype, the trait is said to show incomplete penetrance. If all carriers show the phenotype, then the trait is said to have complete or full penetrance. For example, familial cases of central precocious puberty show reduced or incomplete penetrance.

Expressivity refers to the severity of the phenotype in different individuals carrying the same disease-causing genotype. If the severity of the phenotype differs in people with the same disease-causing genotype, the phenotype shows *variable expressivity*. Disease expressivity includes age of onset, rate of progression, severity and the manifestation of other comorbidities. There are numerous examples of studies of non-identical twins who share the same environment and carry the same disease-causing genotype yet display distinct phenotypes.

This suggests that genetic factors are acting as modifiers of the phenotype. The effect of one gene or allele on the phenotypic outcome of a second gene or locus is termed epistasis, genetic interaction, digenic inheritance, oligogenic inheritance or genetic modifier. Although these are essentially synonyms, there are important distinctions that can be drawn.

If a mutation in the primary gene is both necessary and sufficient to cause disease, the presence of an allele at a second gene has a purely modifying role on the severity of the phenotype. Digenic or oligogenic inheritance is termed when alleles at two (di-) or more (oligo-) genes are required to manifest the pathology. In practice this distinction is often blurred. It is important to note that variability in the expression of the phenotype could also be due to environmental factors, perhaps interacting with genetic variants. Examples of genetic modifiers include variation in an interacting protein partner or, if the protein is a DNA-binding transcription factor, variation in target binding sequences. An example of variable expressivity is the phenotype associated with mutations in the *NR5A1* gene. Here, mutations involving the same amino acid change are associated with 46,XY complete gonadal dysgenesis in some individuals and infertility in others. Digenic and, more rarely, oligogenic inheritance has been reported in individuals with central hypogonadotropic hypogonadism (CHH). Where the causal mutation has been identified, over 80% of patients with CHH have a monogenic cause but ~12% have digenic and 2.5% oligogenic inheritance.

Human Populations and Genetic Variation

Overview of Human Genetic Variation

Mendelian phenotypes result from mutations that alter the function, localization and/or the presence of a protein. Even though protein-coding sequences comprise only around 2% of the human genome, linkage analyses on pedigrees with various disorders have shown that the vast majority of disease-causing mutations are variants that directly impact protein expression or function. This excludes ascertainment bias. Overall, clinically recognized Mendelian phenotypes occur in ~0.4% of all live births and 8% of live births have a genetic disorder recognizable by early adulthood. The Human Genome Project and subsequent *annotation* efforts have established that there are around 19,000 predicted protein-coding genes in humans. The consequences of germline mutations (single nucleotide variants [SNVs] and copy number variants [CNVs]) are known for more than 2300 of these genes. Around 3300 genes have been implicated in Mendelian disorders and this figure is growing at around 300 per year.

Humans are 99.9% identical with respect to their DNA sequence. A typical human genome from an apparently healthy individual differs from the reference genome at 4.1–5 million sites (>99.9% SNVs or indels) and carries 300–600 non-synonymous mutations that are found in <1% of the general population (minor allele frequency, MAF < 0.01). This includes around 150 mutations that are not (yet) present on any of the public variation databases and that are a combination of *de novo* or family or community-specific DNA variants. All of us inherit about 100 likely loss-of-function or nonsense variants from our parents and around 25–30 variants per genome that have been reported to be associated with rare diseases (ClinVar: http://www.ncbi.nlm.nih.gov/clinvar).

One of the surprises from the large amount of genomic data generated from healthy control populations in recent years, such as the 1000 genomes project, is the relatively high prevalence of mutations that have previously been reported as causing severe disease. This suggests that a combination of incomplete penetrance, a false assignment of pathogenicity or a wide range in the expressivity of the phenotype may be more common features of disease mutations than is generally appreciated.

Genetic variation in the general human population can be interrogated using dbSNP (http://www.ncbi.nlm.nih.gov/SNP) or the Exome Aggregation Consortium (ExAC; http://exac.broadinstitute.org). The Exome Aggregation Consortium (ExAC) data set contains exome sequence data from more than 60,000 individuals with an assigned geographic ancestry. Approximately 60.9% of the samples in the ExAC reference cohort are of European ancestry, compared with 13.7% of South Asian ancestry, 9.6% of Latino ethnicity, 8.6% of African (African American) ancestry and 7.2% of East Asian ancestry.

Allele Frequencies Differ in Different Populations

Although most variants are common across human populations, rare gene variants can show markedly different patterns across different human communities. The 1000 genomes project established that there are several hundred thousand SNVs that show considerable differences in allelic frequencies in geographically and ancestry distinct populations. There are several explanations for this. Local populations may have adapted to their specific environments and genetic variants that facilitated this adaptation were selected by evolution (positive selection), which could explain the high frequency of mutation in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene in individuals of European ancestry. Carriers of *CFTR*

mutations may have had more resistance to cholera and other dehydrating intestinal disorders or are more resistant to contracting tuberculosis.

The demographic history of a population can also have a dramatic impact on allele frequencies in modern populations. Migration can change allele frequency by the process of gene flow, defined as the slow diffusion of genes across a barrier. This usually involves a large population and a gradual change in gene frequencies. The genes of migrant populations with their own characteristic allelic frequencies are gradually merged into the gene pool of the population into which they have migrated. Historically small and/or isolated populations or populations that experienced a population bottleneck can also effect allelic frequencies. Tay–Sachs disease in Ashkenazi Jews is an example, where a Tay–Sachs mutation arose by chance in a small breeding population and led to a *founder effect*.

Chance events can have a much greater effect on allele frequencies in a small population than in a large one. If the population is small, random effects, such as increased fertility or survival of the carriers of a mutation that occurred for reasons *unrelated* to carrying the mutant allele, may cause the allele frequency to change from one generation to the next. This is termed *genetic drift*. Whatever the mechanism, large-scale sequencing projects are showing that disease-causing alleles at relatively high frequencies in specific populations and communities as well as rare variants may be an important contributor to common diseases. This has an important impact on the clinical work-up of a patient, where it is essential to determine the ancestry of the affected individual.

Copy Number Variation (CNV)

Human populations also show extensive structural polymorphism, both deletions and duplications of chromosomal segments and, consequently, in the number of genes in these segments. Approximately two-thirds of the human genome is composed of repeats and 4.8–9.5% of the genome contributes to CNVs. Indeed, CNVs are thought to account for ~1% of the variation between two individuals. In contrast, SNVs are thought to account for ~0.1% of the variation. CNVs can arise both meiotically and somatically and can therefore contribute to variation between identical twins as well as variation between different organs and tissues of the same individual.

Smaller deletions and insertions (typically >50 kb) can be detected by comparative genomic hybridization (CGH) or multiplex ligation-dependent probe amplification (MLPA) analysis. MLPA is a variation of the multiplex polymerase chain reaction. For a short sequence of target DNA, two adjacent probes are designed to contain the forward and reverse primer sequence, respectively. In addition, one or both probes contain a stuffer sequence of which the length can be varied during the experiment. The probes are hybridized against the target DNA and subsequently ligated. Only if ligation happened does a functional PCR strand appear, so that amplification only happens if target DNA is present in the sample. The amount of PCR product is proportional to the amount of target DNA present in the sample, making the technique suitable for quantitative measurements.

Comparative genomic hybridization is a molecular cytogenetic method for analysing CNVs relative to ploidy level in the DNA of a test sample compared to a reference sample. Classically this was performed by differentially labelling a reference and test genome and hybridizing to an immobilized substrate such as a microarray. The fluorescence ratios provide a representation of the relative DNA CNV. Today, this is performed by a combination of hybridization of unlabelled DNA to target oligomers and enzymatic single-base extension to incorporate a labelled nucleotide for assay read-out but this is likely to be superseded by whole genome sequencing (WGS) in the near future.

There are still many hurdles in the clinical interpretation of CNVs. They are relatively common and there are many examples of known pathogenic CNVs exhibiting reduced penetrance and/or variable expressivity. This can result in a more severely affected child, who has inherited a CNV from a seemingly normal parent. The 22q11.2 deletion (del) syndrome is a classic example of this. In general, large, rare recurrent deletions and duplications are straightforward to interpret because of considerable genetic and phenotypic evidence. Typically, in a large multi-centre clinic, 15–20% of cases with developmental delay are associated with diagnostic findings from whole genome chromosomal microarray (CMA) analysis. Among these diagnostic cases, many rare CNVs are detected for which the potential functional significance is unknown and they are referred to as variants of uncertain (or unknown) significance (VUS). It is important to bear in mind that ~100 genes can be completely deleted from the human genome without phenotypic consequences.

The major challenge in this field is the detection and interpretation of small (>1–5 kb) rearrangements. These are generally too small to be detected by conventional microarrays but can be detected by WGS. Current data indicate that each individual carries many thousands of these small CNVs. Because of the absence of information in public databases on these small rearrangements, the interpretation of these small variants in challenging. CNVs are of considerable importance in pediatric endocrinology. A considerable proportion of individuals with

ovotesticular/testicular DSD carry deletions and duplications in non-coding region flanking the *SOX9* and *SOX3* genes. Duplications of Xp22 and deletions of 9p24 are associated with 46,XY gonadal dysgenesis and ~10% of patients with idiopathic short stature carry a disease-causing CNV.

Epigenetics

Epigenetic Mechanisms

The word 'epigenetic' literally means 'in addition to changes in genetic sequence'. The term has evolved to include any process that alters gene activity without changing the DNA sequence. These modifications can be transmitted to daughter cells, reversed and possibly transmitted to future generations (transgenerational epigenetic inheritance). Many types of epigenetic processes have been identified that include methylation, acetylation, phosphorylation, ubiquitylation and sumoylation.

Perhaps the best-known epigenetic process, in part because it has been easiest to study with existing technology, is DNA methylation. This is the addition or removal of a methyl group (CH_3), predominantly where cytosine bases occur consecutively.

As well as DNA methylation, there are other different types of epigenetic information encoded within our epigenome including, but not limited to, the presence or absence of histones on any particular DNA sequence, chromatin remodelling, post-translational modifications of the histone proteins, structural and functional variants of histones and transcription of ncRNAs. In mammals, chromatin is integrated by nucleosomes, which consists of DNA wrapped twice around a histone octamer, each containing two copies of four highly conserved histones: H2A, H2B, H3 and H4. Histone tails on the nucleosome are subject to post-translational modifications of selected amino acids including methylation, acetylation, phosphorylation, ubiquitylation, biotinylation, sumoylation and ADP-ribosylation. These modifications are associated with changes in both activation and repression of gene transcription. Histone modifications (or histone code) serve to recruit other proteins by specific recognition of the modified histone. Recruited proteins then act to alter chromatin structure actively or to promote transcription.

Genomic Imprinting

Genomic imprinting refers to genes that are expressed in a parent-of-origin-specific manner. If the allele inherited from the father is imprinted, it is thereby silenced and only the allele from the mother is expressed. If the allele from the mother is imprinted, then only the allele from the father is expressed. Over 95 human genes have been demonstrated to be imprinted with over 100 other genes predicted to be subject to imprinting (http://www.geneimprint.com).

Imprinting is due to alterations of DNA, such as methylation of cytosine to form 5-methylcytosine or the modification in chromatin of specific histone types without altering the genetic sequence. These epigenetic marks are established, or imprinted, in the germline of the parents and maintained through mitotic cell divisions in the somatic cells of an organism. Thus, the allelic expression of an imprinted gene depends upon whether it resided in a male or female in the previous generation. Imprinted expression can also vary between tissues, developmental stages and species.

The phenomenon of genomic imprinting evolved in a common ancestor to marsupials and eutherian mammals over 150 million years ago. Its evolution apparently occurred because of a parental battle between the sexes to control the maternal expenditure of resources to the offspring. Paternally expressed imprinted genes tend to promote growth, while those genes that are maternally expressed suppress it. Thus, paternally expressed genes enhance the extraction of nutrients from the mother during pregnancy, whereas the maternal genome seeks to limit it. Why mammals use imprinted genes to regulate embryonic and neonatal growth is unclear.

Most gametic imprints can act on clusters of up to 12 genes at once and can span several thousand kilobases of genomic DNA. The majority of genes in any one cluster are protein-coding but at least one is always an imprinted lncRNA, which generally shows reciprocal parental-specific expression compared to the imprinted protein-coding genes. The grouping of imprinted genes within clusters allows them to share common regulatory elements, such as non-coding RNAs and differentially methylated regions (DMRs). When these regulatory elements control the imprinting of one or more genes, they are known as imprinting control regions (ICR).

There is an understanding of the general principles of how the imprinting mechanism operates at imprinted gene clusters, although the details are still unclear. Two major classes of *cis*-acting silencing mechanisms are hypothesized to govern imprinting. These are the insulator model cluster and the lncRNA-mediated silencing model. An insulator is defined as an element that blocks enhancer and promoter interactions when placed between them. For example, at the *H19* locus on the maternal allele, CTCF (a protein that mediates insulator activity) binds to the ICR and blocks the access of *Igf2* and *Ins2* to enhancers shared with the *H19* lncRNA that are located downstream of the three genes. This allows *H19* exclusive access to the enhancers. On the paternal allele, the ICR acquires DNA methylation in the male germline, preventing CTCF from binding to it. Thus, on

the paternal chromosome, *Igf2* and *Ins2* interact with the enhancers and are expressed from this chromosome. The presence of DNA methylation on the paternal ICR leads to methylation of the *H19* promoter and it becomes silenced on the paternal chromosome. LncRNAs may control imprinting by forming a double-stranded RNA between the mRNA and lncRNA and inducing RNA interference (RNAi). This sense–antisense overlap may also result in transcriptional interference of a promoter or an enhancer, which would induce transcriptional gene silencing throughout the cluster.

Some human disorders show a mode of inheritance of the phenotype that indicates an imprinting defect (see 'Uniparental disomy'). Here, the expression of the disease phenotype depends on whether the mutant allele or abnormal chromosome is inherited from the father or from the mother. Classic examples of imprinting disorders include Angelman and Prader–Willi syndromes. Angelman syndrome is characterized by motor dysfunction, intellectual disability, speech impairment, seizures and common features of autistic spectrum disorder. Seventy percent of patients with Angelman syndrome have a segment of the maternal chromosome 15 (15q11.2–q13) containing the *UBE3A* gene deleted. In 11%, Angelman syndrome is caused by a mutation in the maternal copy of the *UBE3A* gene.

Prader–Willi syndrome is characterized by severe hypotonia and feeding difficulties in early infancy, followed by the gradual development of morbid obesity in later infancy or early childhood. Motor milestones and language development are delayed. All individuals have some degree of cognitive impairment and a distinctive behavioural phenotype. Hypogonadism and short stature is present in both males and females. Prader–Willi syndrome is caused by loss of the paternally imprinted region of chromosome 15.

Alterations in genomic imprinting may also play a key role in anomalies of pubertal timing. Families have been identified who carry mutations in the *MKRN3* gene that encodes the makorin RING-finger protein 3, which has ubiquitin-ligase activity. *MKRN3* is a negative regulator of puberty, with loss-of-function mutations resulting in central precocious puberty. *MKRN3* is subject to imprinting and it is expressed exclusively from the paternally inherited chromosome. Where it has been possible to determine, all the affected children inherited the mutation from their fathers.

Transgenerational and Multigenerational Epigenetic Inheritance

Transgenerational epigenetic inheritance involves the transmission of 'information' over multiple generations via the gametes, independent of the DNA base sequence. Transgenerational epigenetic inheritance refers to the transmission of phenotypes over generations that are not due to inherited changes in the primary DNA sequence. A clear distinction needs to be made between inter- rather than transgenerational effects. For example, if a pregnant mother (designated as the filial [F] 0) is exposed to an adverse stimulus, her child (F1) may be affected as a consequence of direct exposure to the same stimulus *in utero*. Since germ cells of the F1 offspring are developing throughout gestation, the grandchildren (F2) are also directly exposed. Effects seen in the F2 generation would be considered multigenerational, whereas effects observed in the F3 generation that had no direct exposure to the original stimulus would be described as transgenerational. When inherited through the paternal lineage, transgenerational inheritance is established by phenotypes transmitted for two generations to the grandchildren (F2).

A major barrier to transgenerational epigenetic inheritance in mammals is germline reprogramming, during which histone variants and their modifications, as well as small RNAs and DNA methylation, are all reset. In mammals reprogramming occurs both in the germline and the zygote immediately after fertilization. Imprinted loci undergo germline reprogramming but not post-zygotic reprogramming. In animals, there are relatively few examples of heritable epigenetic variation at individual genes but there are many examples of quantitative epigenetic traits that appear to respond to environmental, and especially nutritional, cues experienced by former generations. Exposure to an olfactory cue early in development of the nematode affects behaviour when encountering the chemical in adulthood and this behaviour can be transmitted over more than 40 generations. There is no convincing evidence of transgenerational inheritance in the human.

Advances in Genomic Analysis

Familial Linkage Analysis and GWAS Studies

Linkage refers to the association of genes or regions on the same chromosome. Regions (loci) that are located nearer to each other on the chromosome have a lower probability of being distributed on to different chromatids during chromosomal crossover. These loci are more likely to be inherited together and are therefore said to be genetically linked. Linkage studies are carried out to establish if the genetically linked regions are associated with a trait, particularly a disease one. The frequency at which two loci segregate during homologous chromosomal crossovers is defined as recombination frequency (θ) and is measured in *centimorgans* (cM). The recombination frequency is 50% due to independent assortment when two loci are located distantly on the

same chromosome or are on different chromosomes. The linked loci are less likely to assort independently. A higher frequency of recombination thus suggests a larger distance between the two loci so recombination frequency can be used to create genetic maps.

Distances between linked loci are calculated on the basis of the logarithm of odds (LOD) score (to the base of 10). An LOD score of 3 or more indicates linkage because an LOD score of 3 for 2 given loci means that the odds are 1000 to 1 in favour of them being linked. When the frequency of segregation of their alleles is higher or lower than expected if the loci assorted independently, the loci are said to be in linkage disequilibrium (LD), which is defined as the non-random association of alleles at different loci. This could be produced by any of the following factors: epistatic natural selection, mutation, random drift, genetic hitchhiking or gene flow. LD is dependent on and may be influenced by multiple factors including linkage, recombination frequency, mutation rate, selection and population structures. Analysing LD on defined loci can give an insight into evolutionary and demographic events as well as help map loci associated with quantitative characters and inherited diseases.

With recent advances in technologies, there has been an increase in association studies for complex diseases, either instead of or in addition to linkage studies. The genome-wide association studies (GWAS) are a hypothesis-free, phenotype-first, case–control approach where participants are classified first based on the presence or absence of a trait. The genome-wide variants are then assessed using SNP arrays, whole exome sequencing (WES) or WGS methods. If one or more variants segregates at a statistically significant higher frequency with the given trait, they are considered to be associated with it. This is measured by calculating the ratio between the odds of carrying the variants with the trait as against its absence in the absence of the trait, which is defined as the odds ratio (OR). An OR >1 that carries a significant *P* value shows that the variant is linked with the trait.

Linkage studies are based on linkage analysis of large familial cases, whereas association studies are based on multiple affected cases and controls within a defined population that detect linkage disequilibrium. While linkage studies are best suited to monogenic disorders, they can still be applied to multifactorial disease locus mapping and they have been widely used for multiple complex disorders. GWAS are a more powerful method for detecting variants associated with multifactorial disease.

Most human populations consist of several ethnic groups with a recent admixture so GWAS can identify false positives in sub-stratified populations, which is not a risk in linkage analysis. In order to identify variants with highly statistically significant ORs, GWAS require a large number of well-defined case–control populations and clinical phenotypes with the list of all variables. An example of this is the GWAS carried out in Denmark with 1006 hypospadias cases confirmed at surgery and 5486 controls. After replication genotyping of an additional 1972 cases and 1812 controls from Denmark, the Netherlands and Sweden, 18 genomic regions showed independent association ($P < 5 \times 10^{-8}$), which together can predict a 9% increase in the risk of developing hypospadias.

A combination of linkage and GWAS studies can identify the loci potentially associated with a disease but even when applied to hundreds of thousands of individuals GWAS studies failed to explain the major genetic component of any given trait in most cases. The problem of the missing heritability is the fact that GWAS relies on variants that are common to populations. In some of the diseases, *de novo* or rare mutations clearly contribute significantly to disease risk and GWAS do not take into consideration such mutations.

Advances in Nucleic Acid Sequencing

The first generation of automated DNA sequencers was essentially electrophoresis machines that detected the migration of fluorescently labelled DNA fragments using the Sanger sequencing approach. Although this remains the gold standard for sequencing, the process lacked high throughput capacities and remained expensive.

The second generation of sequencing technologies or NGS relied on alternative systems based on DNA clusters, which involve the clonal amplification of DNA on a surface (Figure 1.3). In this procedure precursor bases are blocked at their 3′-ends and each step corresponds to the addition of a single nucleotide, which is labelled with a specific fluorescent dye. After incorporation the machine registers the nucleotide-specific quantum light emitted. A camera takes images of the fluorescently labelled nucleotides. The dye, along with the terminal 3′ blocker, is then chemically removed from the DNA and the reaction can proceed to the next step of synthesis. This is one of the most widely used NGS systems in current use (e.g. HiSeq2000 Illumina) but it can result in artefacts in the final output since image detection is by a charge-couple device (CCD) camera and the approach itself is based on sequencing by synthesis, which can introduce errors into the process.

These can be overcome by third-generation sequencing approaches such as Ion Torrent or nanopore sequencing. The former allows DNA sequences to be detected without the need for precursor nucleotides or camera devices for image detection. The method is based on a semiconductor-based detection system of H+ ions that

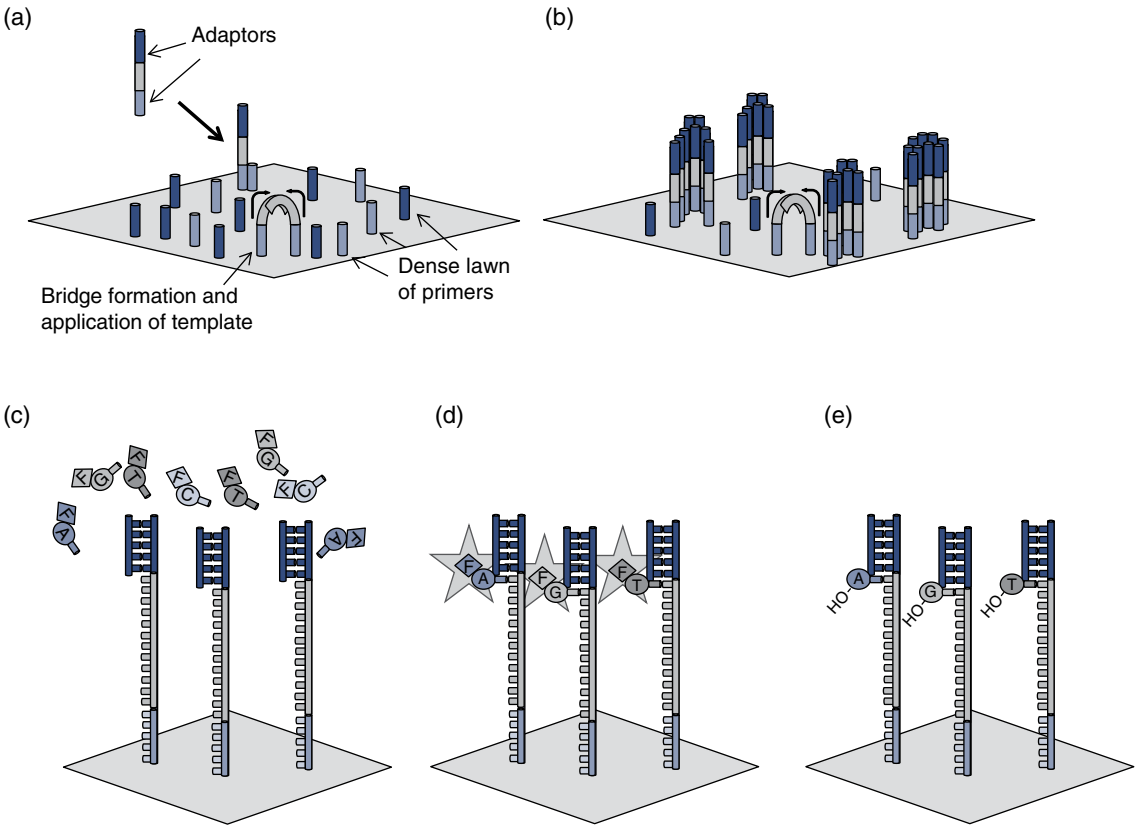

Figure 1.3 Sequencing by synthesis (e.g. Illumina). (a) Randomly fragmented DNA (or exome fragments) is tagged with adaptors (red and blue) and single-stranded DNA fragments (templates) hybridize with complementary primers on a flow cell. Distal ends of hybridized templates interact with nearby primers where amplification can take place. (b) After several rounds of amplification 100–200 million clonal clusters are formed. (c) Fluorophore-labelled terminally-blocked nucleotides hybridize to a complementary base. Each cluster can incorporate a different base. (d) Exposure to laser results in each cluster emitting a colour corresponding to the base incorporated during the cycle. (e) Fluorophores are cleaved and washed from flow cells and the 3′-OH group is regenerated. A new sequencing cycle can then begin.

are a by-product of sequencing by synthesis. Nanopore sequencing uses a protein nanopore set into an electrically resistant polymer membrane. An ionic current is passed through the nanopore by setting a voltage across this membrane. If an analyte passes through the pore or near its aperture, this event creates a characteristic disruption in current. Measurement of the resultant current makes it possible to distinguish between the four standard DNA bases G, A, T and C, as well as modified bases. The error rate for nanopore sequencing is high and, although very useful for example in the detection of pathogens, it is not yet sufficiently robust for human mutation analysis.

NGS Protocols

Over 40 different NGS sample preparation protocols have been published to address different biological questions concerning DNA–protein interactions, chromatin conformation, DNA methylation profiles, transcriptome analysis and RNA–protein interactions. The most commonly used protocols are listed in Table 1.4.

Whole Genome (WGS) and Whole Exome Sequencing (WES)

A patient with a monogenic disease has at most two disease-causing variants, true positive mutations that must be distinguished from the other 20,000 exome variants, in an individual, that are not responsible for the phenotype (false positives). NGS-based testing is becoming a first-line tool in a diagnostic evaluation in suspected monogenetic conditions, including paediatric endocrine disorders. Clinicians and researchers require sequencing protocols that generate accurate and robust genetic data sets for the lowest possible sequencing costs. One of the main costs and considerations for NGS-based genetic testing is the coverage, which is also termed 'depth of coverage' (Figure 1.4). This refers usually to either the number of sequence reads covering a particular position

Table 1.4 Different NGS protocols aimed at addressing specific biological questions.

NGS SP	Comments
RNA analysis	
RNA-Seq	Indicates the presence and quantity of RNA molecules in a biological sample at a given moment in time
ChIRP-Seq	Chromatin isolation by RNA purification detects the locations on the genome where non-coding RNAs and their proteins are bound
GRO-Seq	Global run-on sequencing maps binding sites of transcriptionally active RNA polymerase II
CLIP-Seq	Cross linking and immunoprecipitation sequencing maps protein–RNA binding sites *in vivo*
CLASH-Seq	Cross linking, ligation and sequencing of hybrids that maps RNA–RNA interactions. RNA–protein complexes are UV cross-linked and affinity purified. RNA–RNA hybrids are then ligated, isolated and reverse-transcribed to cDNA and subject to NGS
NET-Seq	Native elongating transcript sequencing maps transcription through the capture of 3′ RNA. The RNA polymerase II elongation complex is immunoprecipitated, RNA extracted and reverse-transcribed to cDNA. NGS of the cDNA allows for 3′-end sequencing of nascent RNA
SHAPE-Seq	Selective 2′-hydroxyl acylation analysed by primer extension sequencing provides structural information about RNA
RIBO-Seq	Detects RNA that is being processed by the ribosome in order to monitor the translation process
RIP-Seq	RNA immunoprecipitation sequencing maps the sites where specific proteins of interest are bound to the RNA within RNA–protein complexes
DNA methylation	
MeDIP-Seq	Methylated DNA immunoprecipitation sequencing is commonly used to study 5mC or 5hmC modification
MethylCap-Seq	Methylation capture sequencing uses proteins to capture methylated DNA in the genome. Sonicated genomic DNA is incubated with tagged MBD proteins that bind methylated cytosines. The protein–DNA complex is then precipitated. NGS provides genome-wide localization of MBD-bound methylated DNA
DNA–protein interaction	
ChIP-Seq	Chromatin immunoprecipitation sequencing maps specific protein-binding sites. In this method, DNA–protein complexes are cross-linked *in vivo*. Samples are then fragmented and treated with an exonuclease to trim unbound oligonucleotides. Protein-specific antibodies are used to immunoprecipitate the DNA–protein complex. The extracted DNA is sequenced, giving high-resolution sequences of the protein-binding sites
DNase-Seq	DNase l hypersensitive sites sequencing is based on DNase I footprinting, which provides accurate location of regulatory proteins in the genome
FAIRE-Seq	Formaldehyde-assisted isolation of regulatory elements is based on differences in cross-linking efficiencies between DNA and nucleosomes or sequence-specific DNA-binding proteins. NGS provides information for regions of DNA that are not occupied by histones
Genome structure and interaction	
Hi-C	Chromosome conformation capture sequencing is used to identify long range inter- and intra-chromosomal interactions in a genome-wide fashion. DNA–protein complexes are cross-linked using formaldehyde. Samples are fragmented and DNA ligated and digested. NGS provides base-pair resolution of ligated fragments. Hi-C identifies the exact positions on the DNA where chromosomes are making contact in the three-dimensional space inside the nucleus
RC-Seq	Retrotransposon capture sequencing is a high-throughput protocol to map and study retrotransposon insertions
Tn-Seq	Transposon sequencing accurately determines quantitative genetic interactions

in the genome or the average number of aligned reads that overlap all positions on the target genome. The latter assumes that reads are distributed randomly across all the target sequences, which is not the case in reality as there are genomic regions that show sequence biases. Compared to the research requirements, sequence-based clinical diagnostic tests require adequate breadth and depth of coverage to ensure that there is a high sensitivity. Greater coverage results in a dramatic reduction in false positive mutations that may be introduced as sequencing errors.

When using WES, a greater average read depth is required to achieve the same breadth of target sequences as that accomplished by WGS. In WGS ~30× average

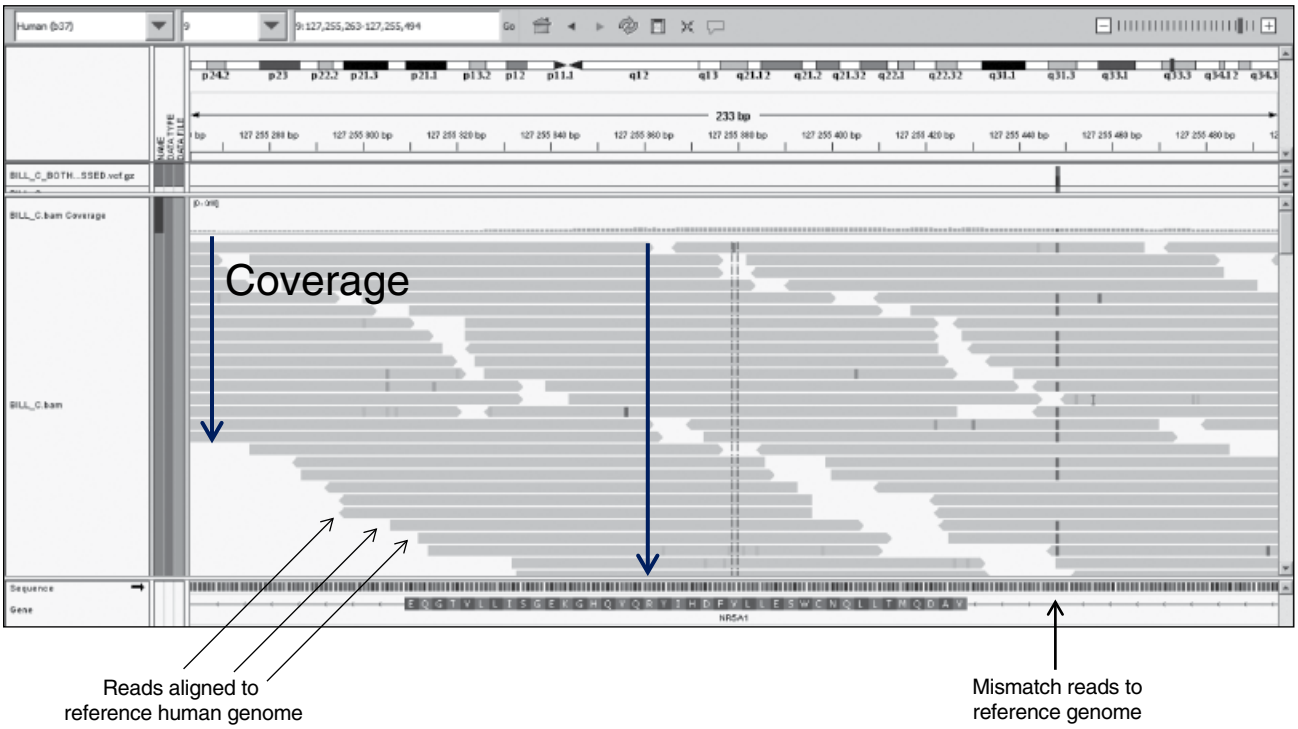

Figure 1.4 Example of NGS exome sequence read alignment data visualized using the Integrative Genomics Viewer (IGV) developed at the Broad Institute that allows for interactive exploration of large genomic data sets. This example shows a coverage plot of chromosome 9q33.3 overlapping a portion of the *NR5A1* gene. Individual aligned reads (gray strips) to the reference genome sequence are highlighted. Reads mismatching the reference sequence are indicated. Descending arrows indicate the coverage or depth of coverage of the sequence at two positions.

depth of coverage across the whole genome is sufficient to detect >99% of homozygous variants and 98% of heterozygous variants.

WGS is the preferred approach to define the entire genetic landscape of an individual compared with WES for several reasons. Without enrichment steps, WGS has the ability to catalogue all variant types in the human genome (large structural rearrangements, CNVs, insertions and deletions [indels] and SNVs) in both coding and non-coding regions. However, an accurate high coverage WGS is still costly compared to a typical ×50 coverage WES, the main drawback of which is the need for enrichment steps before sequencing. Depending on the platform, exome capture technologies show differences in performance and robustness and each of them introduces a sequence bias. If all the genes known to cause a genetic condition have been established, a well-characterized, accurate and sensitive targeted gene panel can overcome these issues.

Variant calling can also be challenging. No single software tool is currently available to identify all variant classes with equal accuracy. Multiple software are available for variant calling and the critical comparison of these tools has highlighted considerable calling inconsistencies. Caution should be exercised in analysing WES data sets including interpreting positive and negative findings with scrutiny, especially for indels, which are notoriously difficult to call in WES. Although there is a considerable effort to improve indel calling, this class of genetic variation is under-represented in exome data sets. Although time-consuming, several variant callers (and/or parameter settings) should be evaluated to optimize the detection of the different variant classes.

Establishing Variant Causality

Dissecting Pathogenic from Non-Pathogenic Variants

One of the most challenging aspects of NGS is determining the causality of a candidate variant. The downstream interpretation of sequence variation data is a formidable barrier to understanding the relevance of genetic variation to the phenotype. In some circumstances, the interpretation can be straightforward if mutations are in the gene with a well-established causal link to the phenotype but the relationship between the variant and the phenotype may be less clear in other cases. Functional assays can be used to determine if the variant affects the function

of the gene product; they can be defined as systematic *in vivo* or *in vitro* experiments designed to determine the biological and biochemical consequence of a mutation on a gene product.

A wide range of functional assays is available and the choice of which to use depends on a number of criteria including the known function of the protein carrying the mutation (e.g. transcription factor, ligand or transmembrane receptor), the location of the mutation in the gene or protein (e.g. DNA-binding domain, protein–protein binding domain, promoter mutation, splice site mutation, etc.) or it may depend on the mutated amino acid itself, since some amino acids undergo specific post-translational modifications.

The time and cost of functional assays means that the biological importance of the many missense mutations in an individual's genome must currently be inferred by computational analysis. The sheer wealth of candidate causal mutations generated by NGS approaches in any human genome can lead to misinterpretation compared to the traditional sequencing of candidate genes by Sanger sequencing. For example, in NGS studies, a *de novo* mutation in a patient with a specific pathology should not immediately imply pathogenicity since *de novo* mutations occur in all healthy individuals.

Several *in silico* approaches can be used to interpret the pathogenicity of sequence variants. Variant-level metrics can be used to predict the biochemical impact of a DNA mutation and thereby prioritize candidate variants for a particular phenotype and the most widely used prediction tools are described in Table 1.5. The CADD method is essentially a meta-annotation tool that is currently the method of choice for mutation analysis. CADD scores correlate with allelic diversity, annotations of functionality, pathogenicity, disease severity, experimentally measured regulatory effects and complex trait associations and they rank known pathogenic variants within individual genomes. However, a general problem with all of these variant-level prediction tools is that, although they can relatively accurately infer the

Table 1.5 Commonly used variant-level prediction tools to understand the potential biological consequences of a mutation.

Tool	Description	URL
SIFT	Predicts whether an amino acid substitution affects protein function. Prediction is based on the degree of conservation of amino acid residues in sequence alignments derived from closely related sequences	http://sift.jcvi.org
MutationAssessor	The functional impact of a mutation is assessed based on evolutionary conservation of the affected amino acid in protein homologues. The method has been validated on a large set (60k) of disease associated and polymorphic variants	http://mutationassessor.org/r3
Align-GVGD	Align-GVGD combines the biophysical characteristics of amino acids and protein multiple sequence alignments to predict where missense substitutions in genes of interest fall in a spectrum from enriched deleterious to enriched neutral. Align-GVGD is an extension of the original Grantham difference to multiple sequence alignments	http://agvgd.hci.utah.edu
MAPP	Evolutionary relationships between the sequences in the alignment are calculated and weights are applied to each sequence to control for the phylogenetic correlation. Each of the 20 possible amino acids per alignment column is represented by the sum of the weights of those sequences carrying the amino acid at that position in the alignment. Scores of physicochemical properties are applied to capture the variation	http://mendel.stanford.edu/SidowLab/downloads/MAPP/index.html
PANTHER	Estimates the likelihood of a particular amino acid-changing SNP to cause a functional impact on the protein. It calculates the length of time (in millions of years) a given amino acid has been preserved in the lineage leading to the protein of interest. The longer the preservation time, the greater the likelihood of functional impact	http://pantherdb.org/tools/csnpScoreForm.jsp?
GERP	A Genomic Evolutionary Rate Profiling (GERP) score measures evolutionary conservation of genetic sequences across species. As the GERP score of a sequence increases, variation within that sequence becomes more rare. A higher GERP signifies an alteration is harmful	http://mendel.stanford.edu/SidowLab/downloads/gerp
PolyPhen2	Prediction is based on a number of features comprising the sequence, phylogenetic and structural information characterizing the substitution	http://genetics.bwh.harvard.edu/pph2
CADD	Predicts the impact of most types of genome variants and integrates the information from many various functional annotations and condenses this information into a single score	http://cadd.gs.washington.edu

biochemical effect of the variant on the protein, they are very poor predictors either of the disease incidence, disease severity or clinical outcome. An emerging consensus has formed that functional inference scores lack the sensitivity and specificity for clinical use. The human genome of any particular individual contains many apparently unambiguous disease-associated variants that very often occur in people with no apparent symptoms.

Gene-level metrics provide information that is complementary to the variant-level metrics. Rather than individual variants, these tools prioritize the genes themselves by using population genetics data for each locus. This provides useful information that can be applied for the further prioritization of variants. Gene-level prioritization tools include genic intolerance, measured by the residual variation intolerance score (RVIS), *de novo* excess (DNE) and the human gene damage index (GDI; Table 1.6). These tools work on the presumption that disease-causing genes are less tolerant to coding variations than other genes. For example, the gene *GCK* encoding glucokinase, an enzyme key to glucose metabolism that helps modulate insulin secretion, has an RVIS score of 28.9% and a deficit in loss-of-function variants (%ExAC_RVIS) among the 7.5% lowest of the human genome. In contrast, the gene encoding the LH receptor, *LHCGR*, has an RVIS score of 74.63% and a deficit in loss-of-function variants among the 30.37% lowest of the human genome. Put simply, this means that the *LHCGR* gene is more tolerant to genetic variation than the *GCK* gene. The underlying logic of gene-level metrics is simple: a rare or novel predicted damaging/deleterious variant in a gene that is not polymorphic in the general population is more likely to be pathogenic than a predicted benign variant in a highly polymorphic gene.

Building a genetic case to support causality may be difficult if the phenotype is rare and/or caused by low-frequency variants in many different genes. Having multiple affected families with the same disease phenotype but different mutations in the same gene is an argument in favour of causality. Several databases exist to provide a platform to improve the sharing of variant data sets and thereby increase the probability of identifying the likely pathogenic mutation associated with a specific phenotype (e.g. PhenomeCentral, https://www.phenomecentral.org; LOVD, http://www.lovd.nl/3.0/home; Gene Matcher, https://genematcher.org). Participants submit both genomic and phenotype data to these sites with the aim of identifying previously uncharacterized disease-associated genes by matching to other comparable cases. Matchmatcher Exchange (www.matchmakerexchange.org) acts as a communication tool between different data sharing sites. The analysis of appropriate ancestry-matched healthy control populations is also required to exclude the possibility that the candidate pathogenic mutation is not a community- or geographic-specific variant. In the publically available variant databases, such as dbSNP138 or the ExAC database, some populations are unrepresented. The absence of genomic data from these and many other groups in the public databases can lead to false genetic associations.

WGS or WES poses serious concerns for *clinical actionability* based on genomic findings because of the sheer quantity of data for each individual or family. The foremost of these are unsolicited or incidental genomic findings, which can be defined as the results of a deliberate search for pathogenic or likely pathogenic alterations in genes that are not apparently relevant to a diagnostic indication for which the sequencing test was ordered. The probability of discovering an incidental finding is estimated between 1 and 8%, depending on the ancestry of the population, and several recommendations have been proposed to deal with these findings and improve the overall interpretation of genomic datasets.

First, it is preferable to use a targeted gene panel as the primary approach in order to avoid unsolicited findings or findings that cannot be interpreted. Second, results should be reported only when they meet a defined threshold for clinical action. Evidence-based actionability

Table 1.6 Commonly used gene-level tools to determine the degree to which a gene can tolerate variation.

Tool	Description	URL/reference
RVIS	Residual variation intolerance score (RVIS) ranks human genes by their deviation from the genome-wide average number of non-synonymous mutations found in genes with a similar amount of global mutational burden	http://genic-intolerance.org
DNE	*De novo* excess (DNE) compares the rate and nature of potential and observed *de novo* mutations per human gene	Samocha KE et al. *Nat Genet*. 2014;**46**:944–950.
GDI	Human gene damage index (GDI) accesses the mutational damage accumulated by each protein-coding human gene in the general population, thus reflecting the combined influences of genetic drift and selection. The GDI tool identifies rare exome variants that are unlikely to be associated with the disease phenotype, whereas RVIS and DNE are better for the detection of true positive mutations that are more likely to be associated with the disease	http://pec630.rockefeller.edu:8080/GDI

protocols are being developed that can help to eliminate subjectivity bias in an actionable decision (www.clingen.org). Third, decisions on the return of data are based on a case-by-case basis and fourth, no data are returned. Guidelines for good laboratory practise for clinical NGS informatics analysis including models for the analysis of sequence variants are continually evolving but Figure 1.5 illustrates a typical clinical actionability protocol for interpreting rare or novel variants associated with disease.

Genome Editing as a Powerful Tool for Establishing Causality

Genome editing is a type of genetic engineering in which DNA is inserted, deleted or replaced in the genome of an organism. The clustered regularly interspaced short palindromic repeats (CRISPRs)/Cas9 system is a powerful technology that enables the editing of parts of the genome by cutting out, replacing or adding parts to the DNA sequence. It is currently the simplest, most versatile and precise method of genetic manipulation that is impacting many fields of biology. CRISPR/Cas9 functions in prokaryotes as an acquired immune system against plasmids and phages where CRISPR RNA (crRNA) together with trans-activating crRNA (tracrRNA) programs DNA binding and nuclease activity of Cas9. For genome editing using CRISPR/Cas9 in mammalian cells, a single guide RNA (sgRNA) is used by fusing crRNA and tracrRNA into a single molecule (Figure 1.6). The Cas9 nuclease relies solely on this 100 nt sgRNA for conferring sequence specificity by forming an RNA–DNA complex between the crRNA portion of the sgRNA and with a region called the protospacer element on target DNA. The tracrRNA portion of the sgRNA interacts with Cas9. Cas9 nuclease then makes a double-stranded break at a site three base pairs from the protospacer adjacent motif (PAM) sequence,

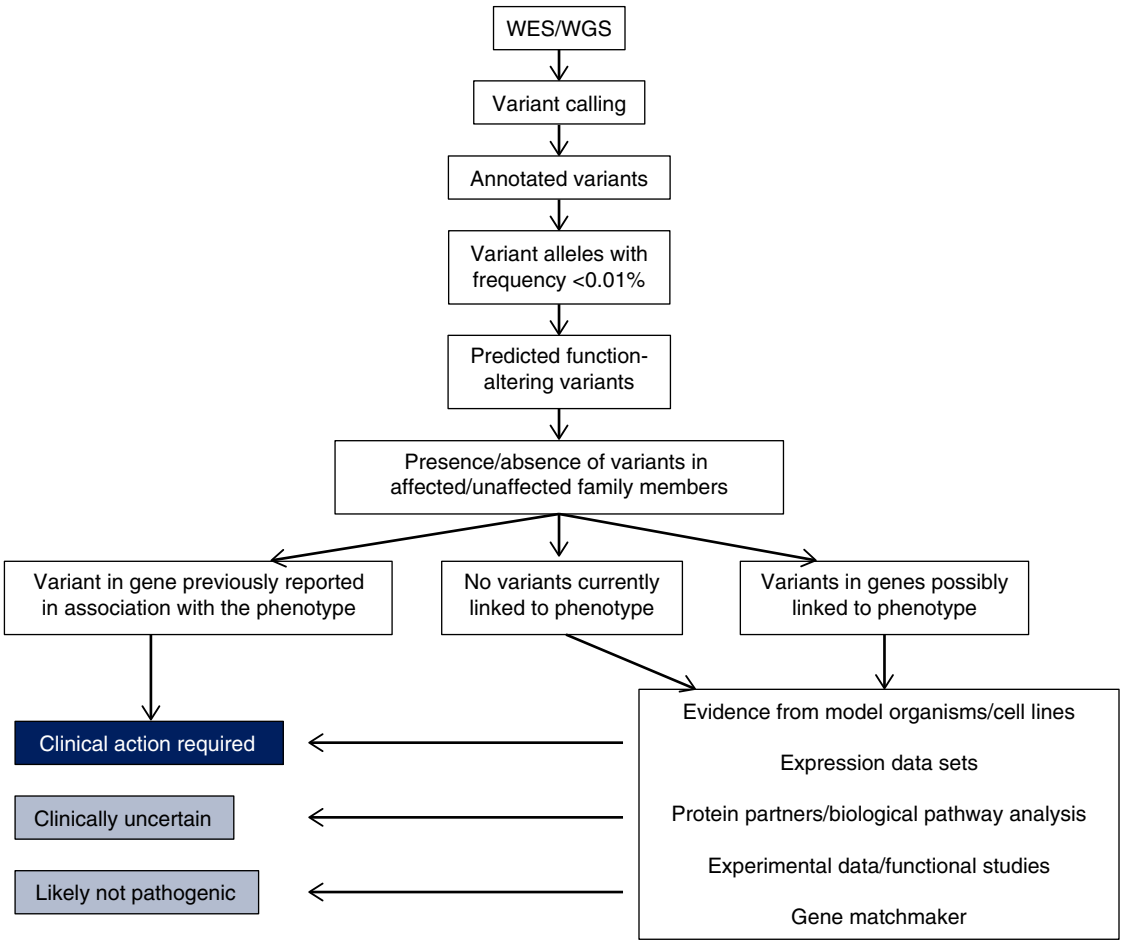

Figure 1.5 An example of a Clinical Actionability Evaluation scheme for either WGS or WES. The protocol is developed to filter variants in order to identify the potentially pathogenic mutation that is associated with the phenotype. Data analysis will lead to one of three conclusions – evidences first that the variant is likely to cause the disease and clinical action is required, second that the clinical involvement of the variant is uncertain based on current knowledge and third that the variant is not likely to be pathogenic.

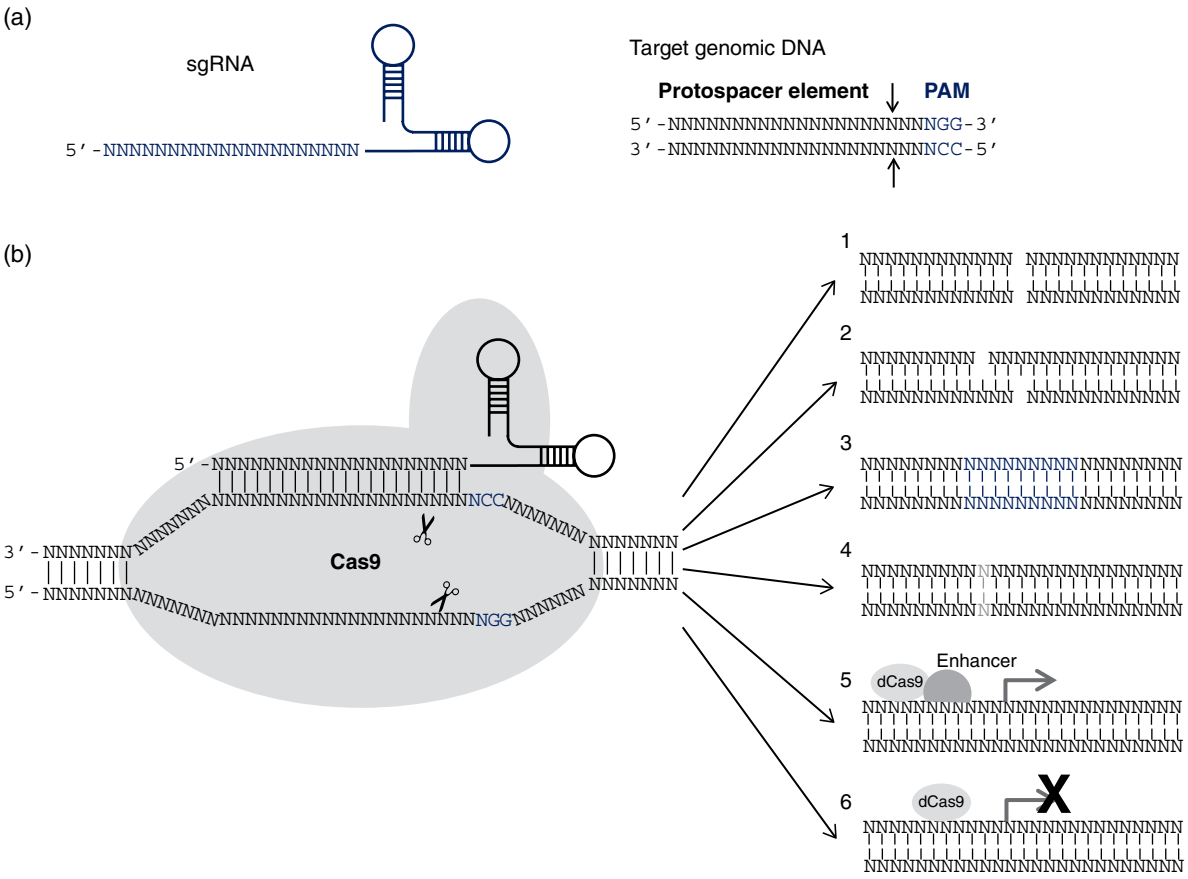

Figure 1.6 Overview of the main CRISPR/Cas9 system and experimental approaches. (a) Left, schematic representation of a single guide RNA, which is a synthetic RNA molecule resulting from the fusion of crRNA (red) and the scaffold tracrRNA (blue). Right, the target DNA sequence. This consists of a protospacer element of 20 nucleotides (black) immediately upstream of the protospacer adjacent motif (PAM), NGG (in red). The arrows indicate the position of the double-stranded break created by the Cas9 nuclease. (b) Left, schematic representation of the CRISPR/Cas9 sgRNA system at the target DNA. Right, different types of genome manipulation are represented. (1) Double strand break. (2) Single-stranded breaks using a modified Cas9 termed Cas9 nickase. Two adjacent sgRNAs targeting different strands will lead to nicks in the DNA and the possibility to create an insertion or deletion. This increases target specificity. (3) It is possible to tag protein encoding genes with either a fluorescent protein or a protein that acts as a selectable marker for the selection of successful knock-in events, for example. (4) Introduction of a single point mutation. (5) A catalytically inactive Cas9 (dCas9) fused to a transcription activator peptide can induce or increase transcription of a gene with cutting the DNA. (6) CRISPR/dCas9 can sterically repress transcription by blocking transcriptional initiation or elongation. Fusing a repressor domain to dCas9 allows transcription to be further repressed by inducing heterochromatinization. (*See insert for colour representation of the figure.*)

which is NGG in the case of Cas9 derived from *Streptococcus pyogenes*, currently the most commonly employed Cas9.

CRISPR/Cas9 nucleases from other species use different PAM sequences and can cover virtually every nucleotide within genomes. CRISPR/Cas9-mediated DNA double-stranded breaks (DSBs) are repaired through either the non-homologous end joining (NHEJ) repair process or the homology-directed repair (HDR) pathway. NHEJ repair often leads to small insertions or deletions (indels) at the targeted site, while HDR pathway leads to perfect repair or precise genetic modification. Various genetic modifications can be achieved through these two DNA repair pathways.

The NHEJ-mediated DNA repair pathway can be exploited to generate null mutation alleles, for example, mouse knockouts, whereas the HDR system is useful in producing precise insertions (knock-ins) of exogenous DNA molecules to create modifications to the genome. A modification of the CRISPR/Cas9 system is used to generate a specific point mutation. In this situation, the resolution of the DSB by homology-directed repair is facilitated by the co-injection of an HDR template. In this case, the HDR template consists of a 200 nucleotide-long

single-stranded DNA (ssDNA) molecule that contains the desired mutation flanked by two homology arms in addition to sequence modifications enabling detection of the altered locus and preventing subsequent cleavage of the repaired locus by Cas9.

Modifications of this system are also very useful for studying transcriptional regulation. A deactivated Cas9 (dCas9), an RNA-programmable DNA-binding protein, can be generated by mutating two key residues within its nuclease domains. dCas9 will bind but not cleave target DNA sequences and can be used to localize transcriptional activators or repressors to specific DNA sequences in order to modulate transcriptional activation or repression. Because of the ease of use, the CRISPR/Cas9 system has swiftly become the most commonly used tool for efficient genome editing of bacteria, plants, cell lines, primary cells and tissues. Due to its precision, this technology can be used to recapitulate various disease-causing mutations seen in human cell lines and can be useful in correcting mutations leading to gene therapy.

The Age of Precision Medicine

The term *precision medicine* is defined as prevention and treatment that takes individual variability into account. It aims to optimize the effectiveness of disease prevention and treatment and minimize potential side effects by considering an individual's specific pattern of genomic variability as well as lifestyle and environmental influences. This term incorporates previously used phrases such as personalized medicine, stratified medicine and targeted therapies. NGS technologies and protocols, as well as advances in cell and gene therapies, are acting as major drivers in precision medicine, especially in cancers and rare diseases.

Although diagnosing a disease and treating it can be considered a simple form of precision medicine, there is now a more refined view of this concept that encapsulates many of the topics in this chapter. Precision medicine can now be used broadly across a large number of disorders due to the creation of large-scale biologic databases (such as databases of human genetic and genomic variation, Table 1.1) and a variety of high-throughput methods for characterizing patient biomarkers of disease (i.e. *proteomics*, *metabolomics*, genomics, transcriptomics). All of these data must be coupled with significant advances in the computational tools (*bioinformatics*) needed for analysing the massive amounts of information generated by the technologies. The advent of precision medicine has resulted in increased efforts to characterize and standardize the description of the patient's phenotype and has generated the field of *phenomics*, generally defined as the measurement of the phenome (the physical and biochemical traits of an organism) and how it changes in response to genetic mutation and environmental influences.

This integration of large -omic datasets is gradually transforming medical practice. Most ongoing research and clinical programs in cancer treatment are based on precision medicine. For several rare diseases, such as familial hypercholesterolaemia, haemophilia and spinal muscular atrophy, approaches using RNA interference are now reaching regulatory approval or late stage clinical development. After initial setbacks gene therapy is gaining momentum with regulatory approval by the European Medicines Agency for targeting the LPL gene in individuals with familial lipoprotein lipase deficiency and retroviral-mediated gene transfer in children with ADA-SCID.

Clinical Guidelines

With the advent of precision medicine, there is an increasing awareness of the need to provide detailed, accurate and, if possible, an agreed standardized description of the complete phenotype of a patient. This is essential in the current scenario where large amounts of genomic data are being generated (e.g. initiatives such as the UK 100,000 Genomes Project), which can be challenging to interpret. A number of guidelines should be considered when the affected child and family seek medical advice.

- Thorough clinical and biochemical evaluation of the patient and a detailed family history including a complete pedigree chart of the extended family as well as information on ancestry must be gathered. This may have to be repeated on different occasions because there may be age-related expressivity (e.g. early menopause associated with *NR5A1* mutations). If a genetic disorder is established, information from the pedigree will reveal carriers and/or individuals at risk for developing the disease. This information is essential for genetic counselling and potential early clinical intervention for those at risk of developing the disease.
- If genetic screening is an option, parental informed consent is required for testing the child. In some circumstances, a specific gene or targeted gene panel may be sufficient to identify the pathogenic mutation, at least in a proportion of cases. Molecular cytogenetics may reveal a CNV associated with the phenotype but, in many pediatric endocrine conditions, such as disorders of sex development, idiopathic short stature or hypopituitarism, the genetic causes are unknown in a majority of cases. In these situations, an exome or whole genome sequence may be proposed, usually in a research environment. If multiple individuals are

affected, they should be sequenced. If there is only the affected child, then the parents should be included if possible.

- Interpretation of genome-wide data can be challenging. A clinical actionability protocol can be followed (Figure 1.6) in association with web-based tools for prioritizing the gene or specific mutation (Tables 1.4 and 1.5). The presence or absence of the candidate mutation in other family members should help interpret the pathogenicity of the candidate mutation. The American College of Medical Genetics and Genomics (ACMG; https://www.acmg.net) and the European Society of Human Genetics (ESHG; https://www.eshg.org) provide regular and updated guidelines for reporting genome variants.
- If causality has not been established, further research involving functional and animal studies should be considered. Sharing information on the candidate variant and associated through gene/mutation matchmaking sites should be considered to strengthen the genetic evidence for causality.

Acknowledgments

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Glossary of Terms

3′-end or flanking region – The 3′-end of a strand terminates at the hydroxyl group of the third carbon in the sugar ring. The 3′-flanking region is a region of DNA that is not copied into the mature mRNA. It often contains sequences that affect the formation of the 3′-end of the message. It may also contain enhancers or other sites to which proteins may bind and may be important for regulation of gene expression.

5′-end or flanking region – Designates the end of the DNA or RNA strand that has the fifth carbon in the sugar ring of the deoxyribose or ribose at its terminus. The 5′-flanking region of a gene often denotes a region of DNA, which is not transcribed into RNA. The 5′-flanking region contains the gene promoter and may also contain enhancers or other protein binding sites. The region is important for normal regulation of gene expression.

Allele – Classically an allele is an alternative form of a gene that is expressed in the phenotype. However, in next-generation sequencing (NGS) an allele is one form of a sequence variant that occurs in any position on any chromosome, or a sequence variant on any sequence read aligned to the genome. In some cases, the term allele is used interchangeably with the term genotype.

Alternative splicing – A process by which exons or portions of exons or noncoding regions within a pre-mRNA transcript are differentially joined or skipped, resulting in multiple protein isoforms being encoded by a single gene.

Annotation – The process of identifying the locations of genes and all other elements in the genome as well as their functions. This may include the likely functional impact of variants.

Argonaute – Protein family that plays a central role in RNA silencing processes, as essential catalytic components of the RNA-induced silencing complex (RISC). RISC is responsible for RNA interference (RNAi). Argonaute proteins bind different classes of small non-coding RNAs. These RNAs guide Argonaute proteins to their specific targets through sequence base pairing, which then leads to mRNA cleavage or translation inhibition.

Autosome – Any nuclear chromosome other than the sex chromosomes.

BAM file – BAM is a binary sequence file format that uses BZGF compression and indexing. It is the binary compressed version of the sequence alignment/map (SAM) format, which contains information about each sequence read in an NGS data set. This includes its alignment position on a reference genome, variants in the read versus the reference genome, mapping quality, and the sequence quality.

Bioinformatics – Computational analysis and storage of biological and experimental data, widely applied to genomic studies.

Biotinylation – Refers to the process of covalently attaching biotin to a protein, nucleic acid or other macromolecules.

Clinical actionability – The ability to use genomic data to change clinical management or therapy.

Coverage – The number of sequence reads covering a particular position in the genome or the average number of aligned reads that overlap all positions on the target genome.

Chromatin – The protein–DNA complex that makes up a chromosome. The state of chromatin – open or closed – usually determines whether a gene is expressed or not.

Cis – Refers to the relationship between two sequences that are on the same chromosome.

Comparative genomic hybridization (CGH) – A fluorescence hybridization technique, which measures the relative content of a specific DNA region or regions between a test and reference DNA sample. Usually performed on a microarray of predefined oligonucleotides (aCGH).

Compound heterozygote – A genotype with two different alleles at the same locus.

Copy number variant – Refers to a variation in DNA sequence defined by the presence or absence of a DNA segment. By convention this usually refers to segments of 200 bp to 2 Mbp.

CRISPR/Cas9 – A technique commonly used for performing genome editing.

***De novo* sequencing –** The sequencing of the genome of a new, previously unsequenced organism or DNA segment. This term is also used whenever a genome (or sequence data set) is assembled by methods of sequence overlap without the use of a known reference sequence. *De novo* sequencing might be used for a region of a known genome that has significant mutations and/or structural variation from the reference.

Dominant – Refers to the member of a pair of alleles that is expressed in the phenotype of the organism, while the other allele is not, even though both alleles are present. It is the opposite of recessive.

Dominant negative mutation – A mutation that dominantly affects the phenotype by means of a defective protein or RNA molecule that interferes with the function of the normal gene product from the same cell.

Enhancer – A DNA sequence that acts in cis to modulate gene transcription. May be located several 100s of kilobases upstream or downstream of a gene.

Epigenetics – The study of how alterations to chromatin structure, rather than DNA sequence itself, can impact heritable characteristics. The sum total of chemical changes to DNA and its associated histone proteins is sometimes known as the epigenome.

Eukaryote – Refers to any organism whose cells contain a nucleus and other organelles enclosed within membranes.

Exome sequencing – Technique for enriching and sequencing most or all of the protein-coding gene segments (exons) in a genome.

Exon – A transcribed region of a gene that is present in mature messenger RNA.

Expressivity – Refers to the extent to which a genetic defect is expressed.

FASTQ file – A text file format for NGS reads that contains both the DNA sequence and quality information about each base.

Founder effect – Refers to a variant that was founded in a population by a small ancestral group or individual.

Frameshift mutation – A deletion or insertion in the coding sequence of a gene that is not a multiple of three. Changes the reading frame of the sequence downstream from the mutation. Usually creates a downstream nonsense codon.

Gain-of-function mutation – Usually refers to a mutation that results in the increase of the biological function of the normal product of a gene. In some circumstances it refers to a mutation that creates a new biological activity of the gene product.

Gene – A sequence of chromosomal DNA that is required for the production of a functional product. Gene symbols are written in italics.

Gene conversion – The process whereby, during meiosis, one allele in a heterozygote is converted to the other by a process of mismatch repair.

Gene silencing – Refers to the ability to prevent the expression of a certain *gene. Gene silencing* can occur during either transcription or translation.

Genetic drift – Refers to the random changes in the frequency of an allele in a small population.

Genome – Totality of genetic information belonging to a cell or an organism.

Genome editing – A technology that allows particular mutations to be introduced into a genome with high specificity and efficiency.

Genomic medicine – Refers to a medical discipline that involves using genomic information about an individual as part of their clinical care and the health outcomes and policy implications of that clinical use.

Genotype – Genetic constitution of an individual cell or organism.

Haploinsufficiency – Refers to a mechanism of action to explain a phenotype when a diploid organism has lost one copy of a gene and is left with a single functional copy of that gene. This contrasts with hemizygosity, which refers to the absence of one of two copies of a gene. Hemizygosity describes the genotype, whereas haploinsufficiency is a mechanism that may have caused the phenotype.

Hemizygous – Refers to the genotype of an individual with only one copy of a chromosome, chromosome segment or gene. Often used when describing X- or Y-linked genes in a male.

Heritability – Refers to the fraction of the total phenotypic variance that is due to genotypic differences.

Heteroplasmy – Refers to the presence of more than one type of mitochondria DNA in the mitochondria of a single individual.

Heterozygote – Refers to an individual or a genotype with two different alleles at a given locus.

Histone proteins – Form the beads (nucleosomes) around which DNA is wrapped in the chromatin that makes up chromosomes.

Homologue – Refers to one of two or more genes that are similar in sequence as a result of derivation from a common ancestral gene.

Homologous chromosomes – Two chromosomes, one of paternal origin and the other of maternal origin, that are identical in appearance and pair during meiosis.

Homoplasmy – Refers to the presence of only one type of mitochondrial DNA in the mitochondria of a single individual.

Homozygous – Refers to an individual or a genotype with identical alleles at a given locus.

Imprinting – Refers to the different expression of alleles depending on the parent of origin of the allele.

In silico – Refers to an analysis performed on computer or via computer simulation.

Indels – Insertions or deletions in one DNA sequence with respect to another. Indels may be a product of errors in DNA sequencing, the result of alignment errors or true mutations in one sequence with respect to another. In NGS, indels are detected in sequence reads after alignment to a reference genome.

Intron – Refers to a segment of a DNA or RNA molecule, which does not code for proteins and interrupts the sequence of genes.

Knockout mouse – A mouse completely lacking a particular gene that may model some aspect of a human phenotype.

Linkage – Refers to coinheritance of two genetic loci that lie near each other on the same chromosome. The closer together the two loci, the greater the linkage and the lower the likelihood of recombination between them.

Linkage disequilibrium – Linkage disequilibrium (LD) is the condition in which the haplotype frequencies in a population deviate from the values they would have if the genes at each locus were combined at random. LD between two loci often indicates that they are physically close to each other on a DNA strand.

Locus (plural loci) – Classically refers to the position occupied by a gene on a chromosome. Can refer to any position on a chromosome.

Loss-of-function variant – Variant causing the reduction or complete loss of a gene product, thereby impairing its biochemical function. Most loss-of-function variants are often only predicted with no supporting experimental evidence.

Mendelian disease – A genetic disease determined by a single locus, exhibiting an inheritance pattern that follows the laws of Mendel.

Minor allelic frequency (MAF) – The frequency of the SNP's less frequent allele in a given population.

Mosaicism – Refers to the presence of two or more populations of cells with different genotypes in one individual who has developed from a single fertilized egg.

Modifier gene/locus – A gene/locus that modifies the phenotype associated with mutations in a non-allelic gene.

Mouse model – A genetically altered mouse that models some aspect of a human disease phenotype. This might be because it has a DNA alteration in the same gene that causes a human phenotype, or a related gene.

Mutation – The changing of the structure of DNA, resulting in a variant form that may be transmitted to subsequent generations, caused by the alteration of single base units in DNA, or the deletion, insertion or rearrangement of larger sections of genes or chromosomes.

Next-generation sequencing (NGS) – Any technology that allows the very rapid sequencing of a whole genome or related population of DNA molecules. Also known as deep sequencing. DNA bases are sequenced from many millions of DNA templates in a single reaction volume. The sequences of all templates are determined in parallel (massively parallel sequencing). Can also be used to examine gene expression (RNAseq) and chromatin structure (ChIPseq).

Non-synonymous – Refers to a nucleotide mutation that alters the amino acid sequence of a protein.

Obligate carrier – An individual who may be clinically unaffected but must carry a specific mutation based on the analysis of a pedigree.

Paired-end sequencing – A technology that obtains sequence reads from both ends of a DNA fragment template. The use of paired-end sequencing can greatly improve overall sequencing quality by allowing contigs to be joined when they contain read pairs from a single template fragment.

Palmitoylation – Refers to the covalent attachment of fatty acids, such as palmitic acid, to cysteine and less frequently to serine and threonine residues of proteins. Palmitoylation enhances the hydrophobicity of proteins and can contribute to membrane association. It also plays roles in subcellular trafficking of proteins between membrane

compartments, as well as modulating protein–protein interactions.

Penetrance – The frequency, expressed as a fraction or a percentage, with which a genotype results in a particular phenotype. If only a proportion of people carrying the genotype display the phenotype, the trait is said to show incomplete penetrance. If all carriers show the phenotype, then the trait is said to have complete or full penetrance.

Phenocopy – Refers to the observed result of an environmentally induced, non-genetic alteration of a phenotype to a form that resembles the expression of a known genetic mutation.

Phenotype – The collection of observable or measurable traits of an individual.

Phred score – Widely used in NGS (*vid sup*) to measure sequence quality. Phred assigns a quality score to each base, which is equivalent to the probability of error for that base. The Phred score is the negative log (base 10) of the error probability and therefore a base with an accuracy of 99% receives a Phred score of 20. Lower Phred scores signify poorer quality and hence potentially inaccurate data.

Pleiotropy – Refers to the ability of one gene to influence two or more seemingly unrelated phenotypic traits. A mutation in a pleiotropic gene may have an effect on some or all traits simultaneously.

Polymorphism – A variant that appears in at least 1% of a population. This value is arbitrary and has been established in human genetics by convention.

Promoter – A DNA sequence located at the 5′ of a gene at which transcription is initiated.

Qualitative trait – A trait that is either present or absent in an individual.

Quantitative trait – A quantity that differs among different individuals. Quantitative traits are measurable phenotypes that vary in degree, for example, height, and can be attributed to polygenic effects.

Recessive – Refers to the member of a pair of alleles that fails to be expressed in the phenotype of the organism when the dominant allele is present. Also refers to the phenotype of the individual that has only the recessive allele.

Reference sequence – The formally recognized, official sequence of a known genome, gene or artificial DNA construct. A reference sequence is usually stored in a public database and may be referred to by an accession number or other designation, such as human genome hg19.

RNA editing – Refers to any process, other than RNA splicing, that results in a change in the sequence of an RNA transcript such that it differs from the sequence of the DNA template.

RNA silencing – Refers to the negative regulation of gene expression by non-coding RNAs (e.g. miRNAs). It may also refer to the introduction of a synthetic antisense RNA molecule to silence gene expression.

Sanger sequencing – The method developed by Frederick Sanger in 1975 to determine the nucleotide sequence of cloned, purified DNA fragments based on the selective incorporation of chain-terminating dideoxynucleotides by DNA polymerase during *in vitro* DNA replication. Widely used to validate potential candidate mutations identified by NGS.

Sequence alignment – An algorithmic approach to find the best matching of consecutive letters in one sequence (text symbols that represent the polymer subunits of DNA or protein sequences) with another. Generally sequence alignment methods balance gaps with mismatches, and the relative scoring of these two features can be adjusted by the user.

Sequence assembly – A computational process of finding overlaps of identical (or nearly identical) strings of letters among a set of sequence fragments and iteratively joining them together to form longer sequences.

Sequence read – When DNA sequence is obtained by any experimental method, including both Sanger and NGS, the data are obtained from individual template molecules as a string of nucleotide bases (G, A, T, C). This string of letters is called a sequence read.

Sex-influenced – A trait that is not X-linked in its pattern of inheritance but is expressed differently in males and females.

Sex-limited – Refers to a trait that is expressed only in one sex. The gene that determines the trait is not X-linked.

Sex-linked – Genes or traits on the portions of the X or Y chromosomes that do not recombine.

Single nucleotide polymorphism (SNP) – A polymorphic variation at a single position in a DNA sequence among individuals. If an SNP occurs within a gene, then the gene is described as having more than one allele.

Somatic mutation – An alteration in DNA that occurs after conception.

Sumoylation – Small ubiquitin-like modifier (or SUMO) proteins are a family of small proteins that are covalently attached to and detached from other proteins in cells to modify their function. This process, termed sumoylation, is a post-translational modification that can modify protein function including nuclear–cytosolic transport, transcriptional regulation, apoptosis, protein stability, response to stress and progression through the cell cycle.

Synonymous – A nucleotide substitution that does not alter amino acid sequences.

Transgenic mouse – Sometimes used to refer to any sort of mouse mutant; traditionally, it describes a mouse that has a piece of foreign or altered DNA inserted into its genome (a transgene) that expresses a novel gene product, often at high levels.

Transition mutation – Refers to a point mutation that changes a purine nucleotide to another purine or a pyrimidine nucleotide to another pyrimidine.

Transversion mutation – A mutation caused by the substitution of a purine for a pyrimidine or vice versa.

Ubiquitylation – Refers to the process of attaching ubiquitin, a small protein found in almost all tissues of eukaryotic organisms, to another targeted protein. Ubiquitination can have different effects on target proteins. Most usually it will lead to target protein degradation via the proteasome, but it may also alter the cellular location, affect protein activity and promote or prevent protein interactions.

Uniparental disomy – The inheritance, in a diploid organism, of both copies of a single chromosome from one parent.

Untranslated region (UTR) – An untranslated region (or UTR) refers to either of two sections, one on each side of a coding sequence on a strand of mRNA. If it is found on the 5′ side, it is called the 5′ UTR, or if it is found on the 3′ side, it is called the 3′ UTR.

Variant call format (VCF) – A generic file format that was used by the 1000 Genomes project for storing DNA polymorphism data such as SNPs, insertions, deletions and structural variants, together with rich annotations. VCF is usually stored in a compressed manner and can be indexed for fast data retrieval of variants from a range of positions on the reference genome.

Variant calling – Identifying the nucleotide or structural differences between a sequence of interest and the reference sequence.

Variant effect predictor (VEP) – A widely used tool within ENSEMBL for the functional annotation of variants generated by NGS.

Variants – Differences at specific positions between two aligned sequences. Variants include single nucleotide polymorphisms (SNPs), insertions and deletions, copy number variants and structural rearrangements.

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2

Measuring Hormones

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KEY LEARNING POINTS

- Endocrine diagnoses can rarely be based on hormone values alone.
- Defining a threshold that separates disease from health within a continuum is arbitrary and artificial and is prone to error.
- In the absence of a sensitivity and specificity of 100%, any test result can be unreliable.
- When assay results are unexpected, technical reasons should be considered and excluded.
- The pretest probability for the disease can be increased by restricting the test to individuals with signs and symptoms.
- Choose endocrine tests with the highest sensitivity and specificity for the specific situation.
- Use tests the results of which will be important for the management of the patient.

Introduction

Endocrinology has the advantage over other medical specialties that the object of medical interest, the hormone, can be measured to confirm or exclude a diagnosis by changing the pretest probability of it, to monitor treatment or to conduct research. Hormone concentrations in human fluids reflect only part of the endocrine system as receptor and post-receptor events are not directly observed. Sensitivity to hormones can vary significantly. In addition, autocrine and paracrine effects of hormones are often not reflected by hormone concentrations in blood.

Hormones comprise peptides, polypeptides, proteins, steroids, catecholamines and iodotyrosines, the different chemistries of which require different analytical methods. Peptides are prone to fast degradation, which requires preanalytical preservation; steroid hormones are less immunogenic than peptides but have very similar structures and are, therefore, more difficult to differentiate by immunoassays although they are well assayed by mass spectrometry (MS).

A laboratory report on hormone concentrations suggests objectivity and accuracy but does not supplant the patient history and clinical observations and endocrine diagnoses can rarely be based on hormone values alone. The aim of this chapter is to explain how to use and critically evaluate hormone measurements.

Hormone Assays

The major assay in use is the immunoassay. A hormone is detected because of its immunogenicity by binding to a capture antibody raised against it or because of its affinity for a particular hormone receptor or binding protein. Since the invention of the radioimmunoassay (RIA) in 1959, techniques have considerably improved [1]. New techniques for the production and purification of antibodies and the recombinant expression of proteins used as standards, as well as a diversity of non-radioactive labelling techniques, such as enzyme-generated colours, fluorophores and luminescent molecules, have been developed. In addition, new separation methods, such as antibody-coated microplates, tubes and beads, have revolutionized and simplified immunoassays [2, 3]. Recent automation and multiplex analysis have been introduced, speeding up the time from sample delivery to laboratory reporting and cheapening the cost of endocrine diagnostics. This process included minimization of incubation times, sometimes at the expense of quality.

The principle of the classical competitive immunoassay (reagent-limited assay) is the competition between the hormone of unknown concentration (sample) and a limited amount of labelled hormone (tracer) to bind to a

Brook's Clinical Pediatric Endocrinology, Seventh Edition. Edited by Mehul T. Dattani and Charles G. D. Brook.

Companion website: www.wiley.com/go/dattani/brookcpe7

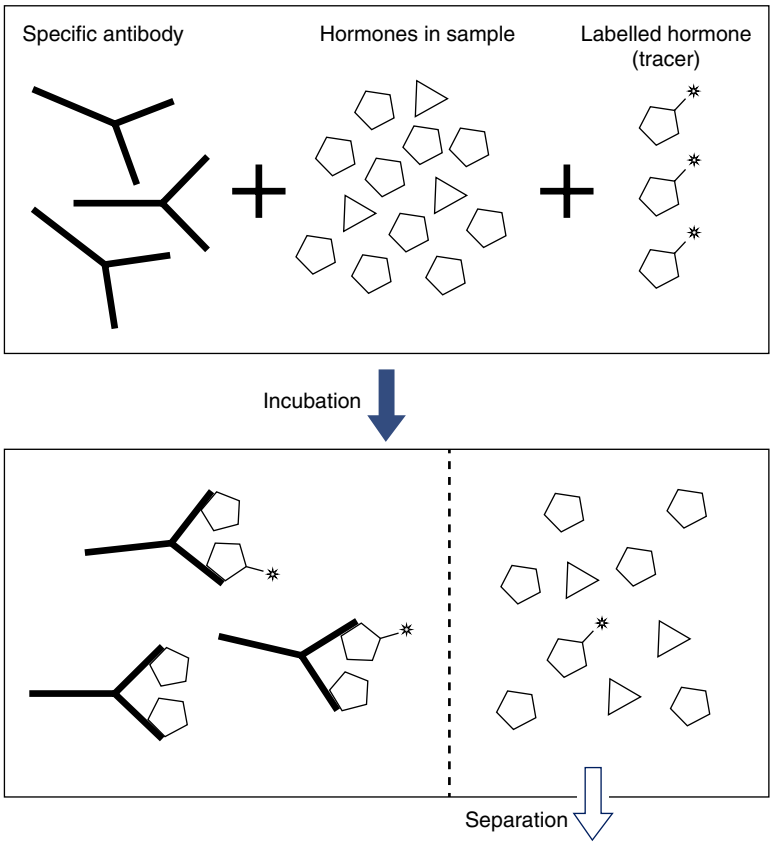


Figure 2.1 The principle of the competitive immunoassay. The hormone in the sample (pentagon) competes with a limited amount of tracer (labelled pentagon) to bind a specific antibody. After separation of the unbound tracer, the signal of the remaining tracer is inversely correlated with the hormone concentration in the sample.

limited amount of specific antibody (Figure 2.1). After the separation of the unbound reactants, the hormone concentration is inversely proportional to the bound tracer. In this type of assay, sensitivity increases with decreasing amounts of the antibody and increasing incubation time. In addition, a relevant determinant of the sensitivity of an immunoassay is the quality of the antibody. A dilution series of the hormone standard (the calibrator) is used for the calibration and calculation of the concentration of the sample, allowing the conversion of the tracer signal into a hormone concentration.

The principle of the non-competitive immunoassay (reagent-excess assay) is the almost complete binding of the hormone of unknown concentration to a high amount of specific antibodies (capture antibodies) fixed on a solid phase. After separation of unbound reactants by washing, incubation with a second labelled specific antibody (detection antibody) enables detection of the hormone in the two-site non-competitive immunoassay, known as a sandwich assay (Figure 2.2). In the one-site non-competitive immunoassay, the capture antibody fulfils the properties of a detection antibody. In non-competitive assays, the amount of hormone is directly proportional to the intensity of the signal. A dilution series of the calibrator enables the conversion of the signal into a hormone concentration. The acronyms of common immunoassays are explained in Table 2.1.

Multiplex technology enables the measurement of multiple hormones simultaneously in one sample. The basic immunoassay technologies frequently used are enzyme-linked immunosorbent assay (ELISA) and enzyme immunoassay (EIA). Multiplex is accomplished either by conjugating antibodies to microsphere beads and mixing different beads together or by spotting different antibodies onto glass, nitrocellulose or well-plates. The advantage of multiplex is the conservation of sample volume, reagents, time and money. Multiplex is well suited for hypothesis-free exploration of large sample numbers or small sample volumes.

A different method of measuring hormones is a MS-based method, e.g. gas chromatography–MS (GC–MS) or liquid chromatography–tandem MS (LC–MS/MS) [4]. Its major use is for the determination of steroid hormone concentrations but catecholamines are also a target. MS-based methods detect hormones by their physical properties of mass and charge (Figure 2.3) [5]. GC–MS is the best method for the identification of nearly all steroid metabolic disorders [6]. Compared with immunoassays, its major advantage is its higher analytical specificity but a major drawback is the time-consuming initial chemical

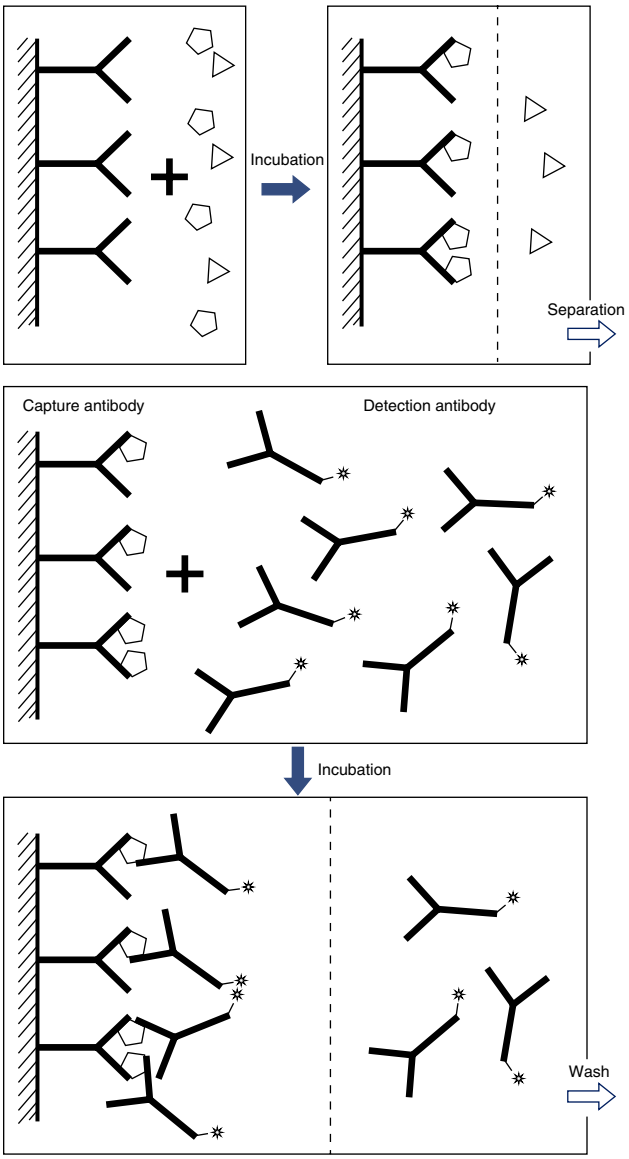


Figure 2.2 The principle of the non-competitive two-site immunoassay. The hormone in the sample (pentagon) binds to an excess of specific capture antibodies fixed on a solid phase. After separation of the unbound antibody, a second so-called detection antibody equipped with a label binds to the hormone. After separation of the unbound detection antibodies, the signal of the remaining detection antibodies is directly correlated with the hormone concentration in the sample.

modification of the sample (derivatization) needed to provide enough volatility to enter the gas phase. LC simplifies the separation as derivatization is no longer necessary but its specificity is lower. GC or LC enables the separation of steroids, whereas MS or TMS allows the highest specificity in determining different steroid metabolites (Figure 2.3). The result is an ion chromatogram, which enables quantification of the sample steroids in comparison with an appropriate standard.

Because of the specificity of GC–MS and LC–MS/MS, steroid hormones, especially sex hormones, should be measured by these methods but this is not always the case [7, 8]. The result of a recent dispute on this topic was a guideline established by a task force from the Endocrine Society emphasizing that the validity and quality of the steroid hormone assay are paramount, regardless of the specific technology, so a specific assay technique was not recommended [9]. Ongoing trials of miniaturization and automation of LC–MS/MS may increase the competitiveness of this method compared with immunoassays and promote the assay of hormones by this method.

Bioassay was the original method of choice for determining hormone concentrations. For example, growth hormone (GH) concentrations were first measured by the hormone's effect on the width of the proximal epiphyseal cartilage of the tibia in hypophysectomized rats (Figure 2.4) [10]. Here, the hormone's biological activity rather than the hormone itself was measured. Bioassays need living animals or cells and are time consuming and labour intensive so they are reserved for standardization of hormone preparations (IRP, international biological reference preparations of the WHO), for drug development and for the detection of rare cases of biologically inactive hormone syndromes or hormone insensitivity syndromes in research laboratories. Bioassays may provide an explanation for discrepancies between the clinical picture of hormone deficiency and a normal or high hormone concentration measured by immunoassays [11].

Assay Validity

The first question is whether an assay measures what it intends to measure. Comparison of an assay in question with a reference, the so-called gold standard assay, in a cohort of relevant patients and healthy controls would be the ideal way to answer this question but gold standards are largely missing in pediatric endocrinology, the only exception being GC–MS for the determination of steroid hormones. As an alternative approach to ensure assay validity, standards have been defined for the accurate description of the quality of an assay [12, 13] thus generating an assay validation report that contains information on the sensitivity (limit of detection), specificity, precision and accuracy of the assay.

Sensitivity describes the performance of an assay at very low concentrations. Assays may give a detection signal even in the absence of hormone so one method of describing analytical sensitivity is the minimally detectable concentration. This limit is frequently defined as the mean + 3 standard deviation (SD) values of at least 20

Table 2.1 Immunoassay acronyms and their meaning.

Acronym	Assay	Label (examples)	Reagents
RIA	Radioimmunoassay	^{125} J (antigen)	—
IRMA	Immunoradiometric assay	^{125} J (antibody)	—
ELISA	Enzyme-linked immunosorbent assay	Alkaline phosphatase	4-Nitrophenylphosphate
EIA	Enzyme immunoassay	Peroxidase	*ortho*-Phenylenediamine
FIA	Fluorescence immunoassay	Fluorescein isothiocyanate	—
CLIA	Chemiluminescence immunoassay	Luminol	Peroxidase, H_2O_2
ECLIA	Electro chemiluminescence immunoassay	Ruthenium	Tripropylamine

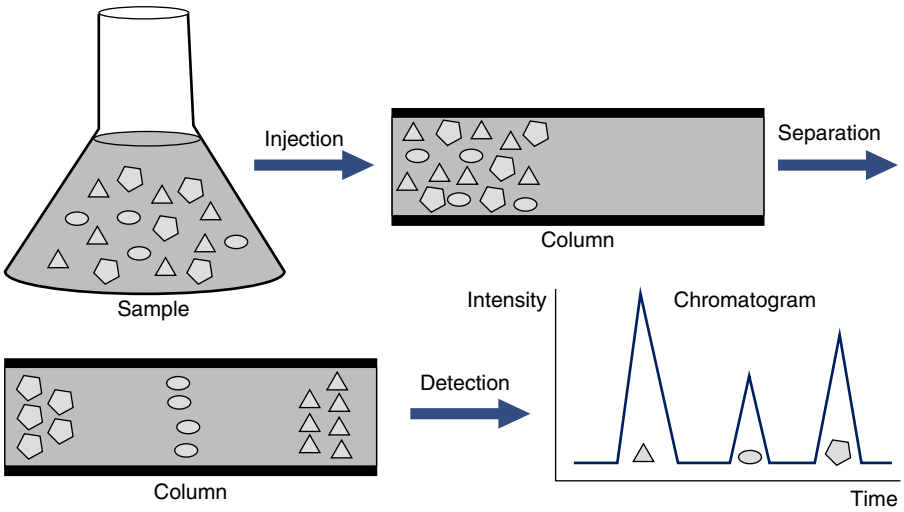


Figure 2.3 Schema of gas chromatography–mass spectrometry (GC–MS). *Source:* Adapted from Sanchez-Guijo et al. [5].

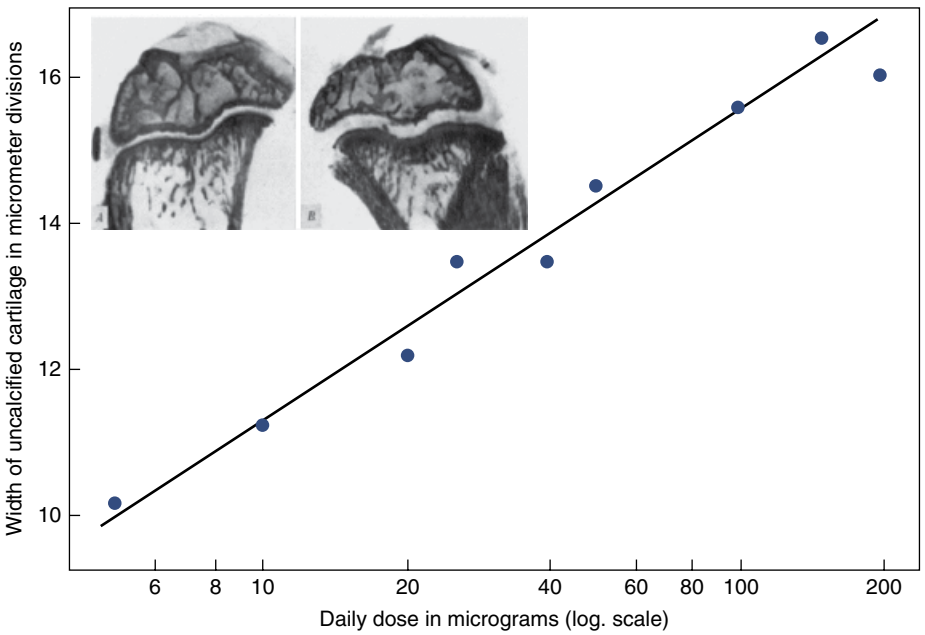


Figure 2.4 Historical bioassay of growth hormone. Hypophysectomized female rats were injected intraperitoneally with an unknown amount of GH for 4 days. Twenty-four hours after the last injection, the rats were sacrificed, and the right proximal tibia was dissected and fixed. The white band of the proliferating zone of the epiphysis was measured under the microscope using a calibrated micrometer eyepiece. The width of the cartilage was directly correlated with the amount of GH injected. *Source:* From Evans et al. [10]. Reproduced with permission of Oxford University Press.

measurements of the null standard which contains no hormone.

A more popular approach to define analytical sensitivity is the so-called limit of quantification or functional sensitivity: it is well known that the lower the concentration of a hormone, the higher the variation in repeated measurements. Accordingly, the limit of quantification is defined by the lowest concentration of the hormone where the intra-assay coefficient of variation (CV) is below 20%. The limit of quantification is specified in the assay report and concentrations below this level cannot be measured and are usually reported as '<*X*', where *X* is the lower limit of detection. Such a report does not prove the absence of the hormone in a sample but it shows a very low, non-detectable concentration.

Differences in assay sensitivities are caused by the quality of the antibodies, the type of the signal and its amplification and the detection method used. For exact quantification of very low hormone concentrations, the assay with the lowest limit of quantification should be chosen.

Specificity requires that an assay antibody should detect only the hormone and nothing else. For some hormones, assay specificity is a tremendous challenge, such as for steroid hormones that have very similar and small structures or for protein hormones that circulate in different isoforms or as subunits, such as GH, hCG or parathyroid hormone. In these cases, it is necessary to define which isoform(s) should be detected by the assay.

Binding specificity is an important quality of the assay antibody. Polyclonal antibodies derived from several B-cell clones are less specific than a monoclonal antibody that detects only one epitope of the hormone. However, even this specificity of a monoclonal antibody for one epitope may not be sufficient to prevent cross-reactivity with other hormones. To improve specificity in sandwich assays, two different monoclonal antibodies are used that detect two separate epitopes on the same hormone. Another way to improve specificity is to separate cross-reactants from the sample by adsorption, solvent extraction or chromatography. Cross-reactivity should be kept to a very low level, documented and specified in the assay validation report. In addition to antibody specificity, other factors, such as binding proteins, autoantibodies against the hormone or heterophilic antibodies, can profoundly influence the specificity of an immunoassay.

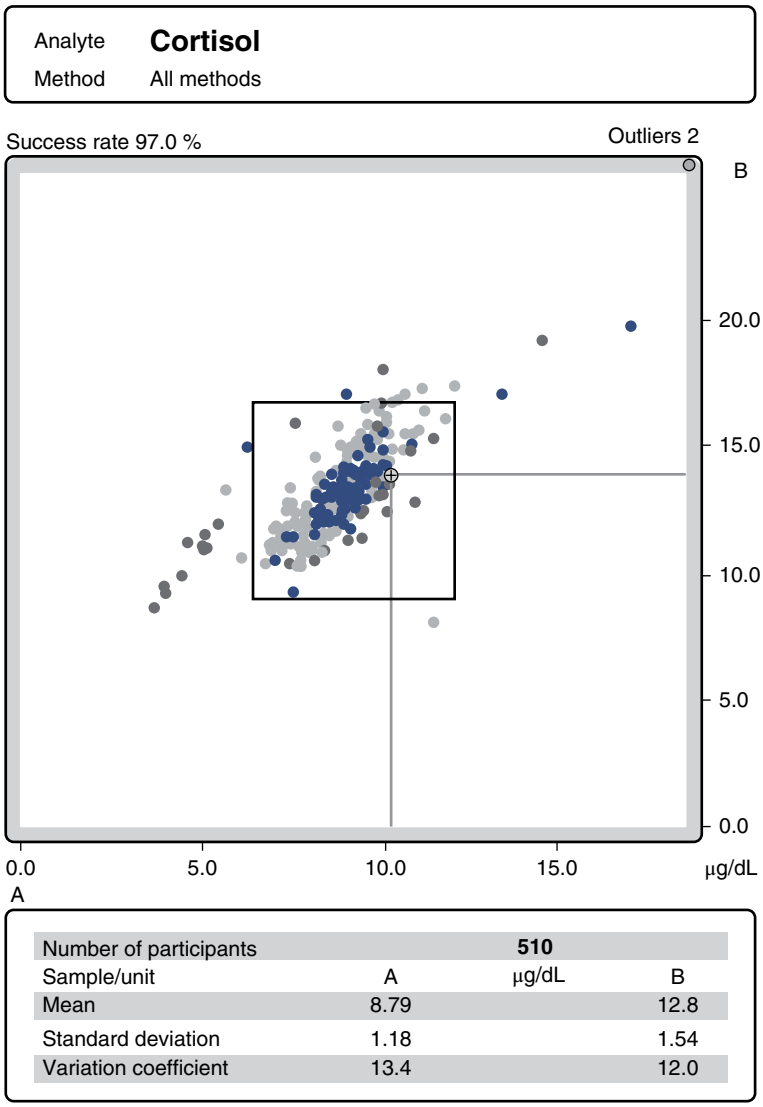
Precision describes the ability of an assay to produce the same value in repeated measurements of a sample. The reproducibility depends on several factors within the assay procedure, including pipetting volumes, manual or automatic pipetting, duration of incubation, washing steps, temperature, reagent stability and quality. Low concentrations of a hormone are less precisely measurable than higher concentrations. Precision is quantified by calculating the intra- and inter-assay coefficients of variation. Two or three quality control samples containing different concentrations, ideally covering the relevant concentration range of the hormone, are measured in duplicate in each assay run. The inter-assay CV is the ratio of the SD to the mean of measurements of 20 different assay runs [3] and should be below 15% at the hormone concentration of interest. The intra-assay variance, based on multiple measurements of the same sample within one assay, should be below 10%. Typically, the inter-assay CV is higher than the intra-assay CV.

Since inter-assay precision is lower than intra-assay precision, it is advisable always to measure samples from longitudinal studies in a single assay run. One should keep in mind that the increase or decrease in a lab value during the follow-up of a patient is always caused partly by the lack of precision of the assay. Unexpectedly high or low hormone values frequently tend to regress to the mean, a statistical phenomenon that can make natural variation in repeated data look like real change [14].

Accuracy describes how close the assay-derived value is to the 'true' value. National inter-laboratory comparison programmes aim to provide quality control to keep hormone measurements of different laboratories and different assays within a tolerable degree of accuracy. Participation in such external quality assessment programmes is mandatory for accredited hormone laboratories. The comparator in use is rarely a gold standard test, such as GC–MS. More often the mean concentration of the measurements from all laboratories taking part in the external quality assessment programme is chosen as the 'true' hormone concentration.

Alternatively, the mean concentration of the measurement from a frequently used commercial assay is taken as the comparator for all laboratories using this specific assay. The results are displayed in a Youden plot that indicates the individual results from two different probes, A and B, of the laboratory, the variation in the measurements from all laboratories and the accepted interval (Figure 2.5). Repeated test failure can be punished by a loss of accreditation for the assay. The Youden plot demonstrates the assay dependence of hormone values: immunoassays from different manufacturers are not harmonized and produce different values from the same sample. This is a major problem in endocrinology that needs to be addressed. Causes include differences in calibrators (standard), antibodies and assay conditions. The weaknesses of external quality assessment programmes are the frequent use of samples with hormone concentrations not representative of children and adolescents and the exclusion of rarely measured hormones.

Accuracy is also proven by control samples included in each assay run so a necessary internal quality control is the entrainment of three standard quality control



Number of participants		510	
Sample/unit	A	µg/dL	B
Mean	8.79		12.8
Standard deviation	1.18		1.54
Variation coefficient	13.4		12.0

Figure 2.5 Youden plot for external quality control. The Youden plot is a graphical technique for analysing inter-laboratory data when each laboratory has assayed two different samples. Here, the cortisol measurement values of probe A (*X* axis) and probe B (*Y* axis) from 510 German clinical laboratories are plotted. Each dot represents one laboratory. The square indicates the accepted range of the two measurements (±30%). The lines indicate the outcome of our laboratory. Laboratories producing outliers should evaluate their assay. *Source:* From http://www.dgkl-rfb.de.

samples with different hormone concentrations in each assay run. For this purpose, the quality control samples must be measured 15–20 times in separate assay runs in advance. The mean ± 2 SD of these measurements is calculated and the target range for each quality control sample is defined. The measured concentrations of the quality control samples need to be within the defined target range to ensure the correct technical performance of the assay run (Figure 2.6). Assay runs with quality control sample results that leave the target range should be repeated until the values of the quality control samples are within the defined confidence interval. Repeated assay failure indicates a technical or operator problem, which must be corrected.

Commercial immunoassays contain biochemical reagents that change with time. Such changes should be reported by the manufacturer to the customer because they can cause abrupt shifts in assay measurements thereby altering the target range for accurate performance.

Technical Pitfalls When Measuring Hormones

When assay results are unexpected, technical reasons are probable and should be excluded. In competitive immunoassays, the amount of signal is inversely correlated with the amount of hormone in the sample and the

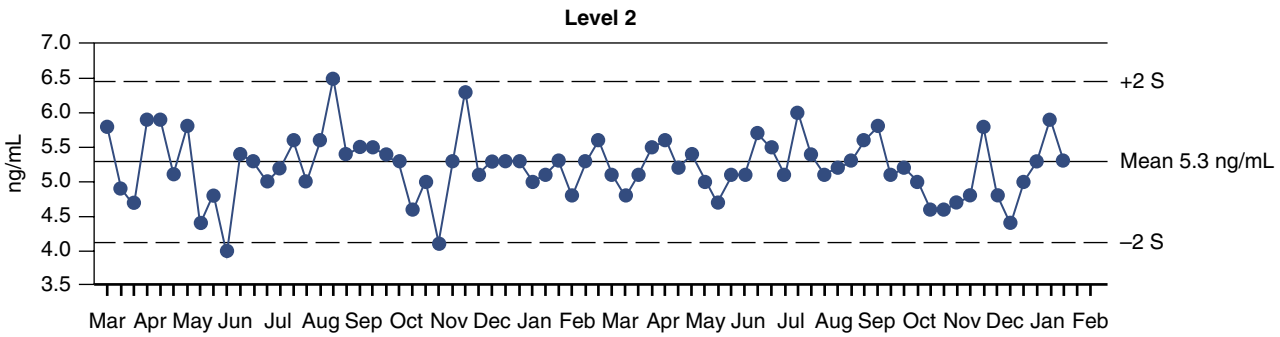


Figure 2.6 Control chart for internal quality control. Three quality control samples with different hormone concentrations are incorporated in any assay run. The results are plotted on the internal control chart. Patient data can be released when the results of the quality control samples are within the defined confidence interval. The figure shows the results of only one of three quality control samples; all internal control results should be within the confidence interval.

hormone concentration is calculated accordingly. Any confounding factor that interferes with antibody binding to the labelled antigen (tracer) or with tracer precipitation causes a low signal and an erroneous apparent increase in the hormone concentration. In non-competitive immunoassays, the amount of signal is directly correlated with the amount of hormone but interference with antibody binding to the hormone can also cause an erroneous signal.

Endogenous autoantibodies, such as thyroglobulin autoantibodies in autoimmune thyroiditis or anti-GH antibodies present in children treated for GHD, can cause falsely elevated values when a competitive assay is used [15]. This may be explained by washing out tracer bound to the human anti-hormone antibodies but, in non-competitive assays, such antibodies can interfere with the antibody–ligand–antibody sandwich to cause falsely low values. In such situations, it is helpful to detect interfering antibodies in the sample by the use of specific assays. In addition, the measurement of a dilution series of the sample may give a hint to the presence of interfering antibodies when the measured signal either does not decrease in a linear fashion or increases.

Heterophilic and human anti-mouse antibodies comprise a group of ill-defined antibodies of the IgM or IgG class [16, 17], which have low affinity and wide reactivity. They were first described as agglutinants of sheep erythrocytes in mononucleosis. They are present in low concentrations in ~40% of blood samples and most have anti-bovine properties possibly induced by drinking cow's milk and eating meat. In individuals with autoimmunity and inflammation, heterophilic or anti-mouse antibodies may be present in higher concentrations and they can cause bridging between the capture antibody and the detection antibody in the absence of hormone in non-competitive immunoassays. This bridging results in a false high or positive result (Figure 2.7).

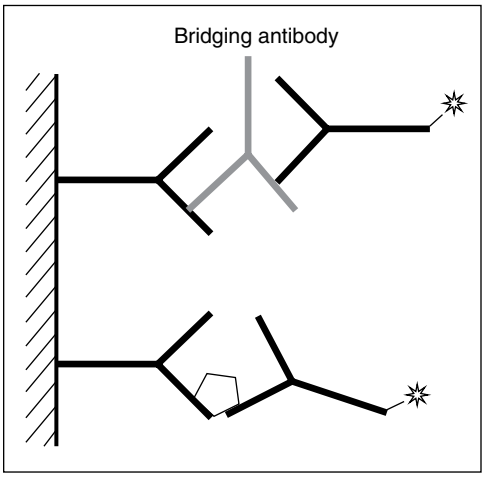


Figure 2.7 Interference by bridging antibodies. Heterophilic antibodies are frequent in human serum and have wide reactivity, albeit with low affinity. The schema illustrates the effect of these antibodies in a non-competitive two-site immunoassay. The heterophilic antibody can build a bridge between the capture antibody and the detection antibody in the absence of the hormone. This bridging results in a falsely high result.

Alternatively, heterophilic antibodies can selectively bind and block only the capture or the detection antibody resulting in a false low or negative result. To avoid such interference, manufacturers of immunoassays enrich most immunoassay reagents with non-immunogenic gamma-immunoglobulins from animal sera, which serve as blocking reagents that allow the binding of the interfering heterophilic antibodies. However, in cases of a Fab–Fab interaction between the heterophilic antibody and the antibody of the immunoassay, such a blocking reagent may not be effective.

Competitive immunoassays are less vulnerable to heterophilic antibodies because the reduction in the amount of capture antibody is rarely sufficient to decrease the signal. Incorrect measurements due to heterophilic or

anti-mouse antibodies have been reported for the measurement of fT4, fT3, TSH, calcitonin, IGF-I, testosterone, LH, FSH and prolactin [16, 17].

The *'Hook effect'* describes a falsely low measurement by an immunoassay in the presence of an elevated amount of hormone that exceeds the highest concentration of the assay calibrator [18]. Such failure occurs mainly in non-competitive two-site sandwich assays when all reactants are given to the reaction tube together. The suggested mechanism is that, after binding to the capture antibody, the remaining free hormone is almost completely bound to the detection antibody, blocking the bridging between the capture and detection antibodies. The washing step that follows eliminates most of the detection antibody resulting in a significant loss of signal and a falsely low sample value (Figure 2.8). Interestingly, the 'Hook effect' was also observed in two-step non-competitive assays. Here, complex interactions between the hormone and the two antibodies are suggested. The 'Hook effect' has been observed for calcitonin, TSH, prolactin and hCG.

Matrix effects describe any characteristic of the sample other than the hormone and are evident when assays validated for serum are used for other fluids, such as saliva, amniotic fluid or pleural fluid. Changes in the assay and its reagents are required to maintain the validity at the same level. It is important that the calibrators are prepared in the same matrix as the sample and the same is true for internal and external quality controls, which is sometimes challenging. Substances released during haemolysis may also interfere with accurate hormone measurements in serum due to colorimetric effects or proteolytic effects by enzymes released from blood cells. Therefore, serum and plasma should always be monitored for the degree of haemolysis and, if possible, assay results should be corrected for the degree of haemolysis.

Binding proteins such as TBG, GHBP or IGFBP3 are potential confounders when measuring hormones because they compete with the assay antibody for binding the hormone. Such interference may be reduced by precipitation, extraction or hydrolysis as well as by the use of high affinity antibodies. In the case of the IGF-I assays, initial acidification of the sample causes dissociation of IGF-I from IGFBPs and a re-neutralizing buffer with the first highly specific antibody and an excess of non-measured IGF-II is added. The IGF-II occupies the IGF-binding sites of the IGFBPs and blocks the interference with the assay.

Clinical Assay Validity

The question whether an assay predicts hormone deficiency, excess or normality is the principal reason for measuring hormones to distinguish between healthy and non-healthy situations. Hormone assays or tests frequently claim to be sensitive or specific for a diagnosis but clinical validation is more important than laboratory results. The promise of distinguishing diseased from disease-free individuals in every case has not been fulfilled [19, 20].

The ideal situation is that a positive test result implies disease and a negative one excludes it (Figure 2.9a) but the real word is more complicated. A positive result can be incorrectly positive and a negative one incorrectly negative (Figure 2.9b, c) [21] because of individual and inter-assay variation, stochastic (random and unpredictable) events, wrong indications, pre-analytic mistakes, sample mix-ups, heterophilic antibodies, analytical

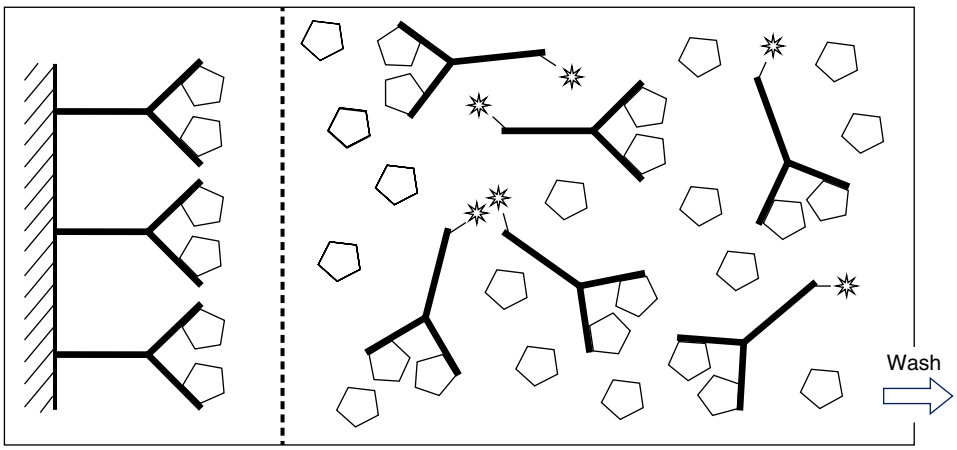


Figure 2.8 The 'hook effect'. A very high amount of hormone exceeding the highest concentration of the assay calibrator can cause false low or negative assay results in non-competitive two-site sandwich assays. The graph shows the complete saturation of all available antibody binding sites, precluding the bridging between the capture and detection antibody.

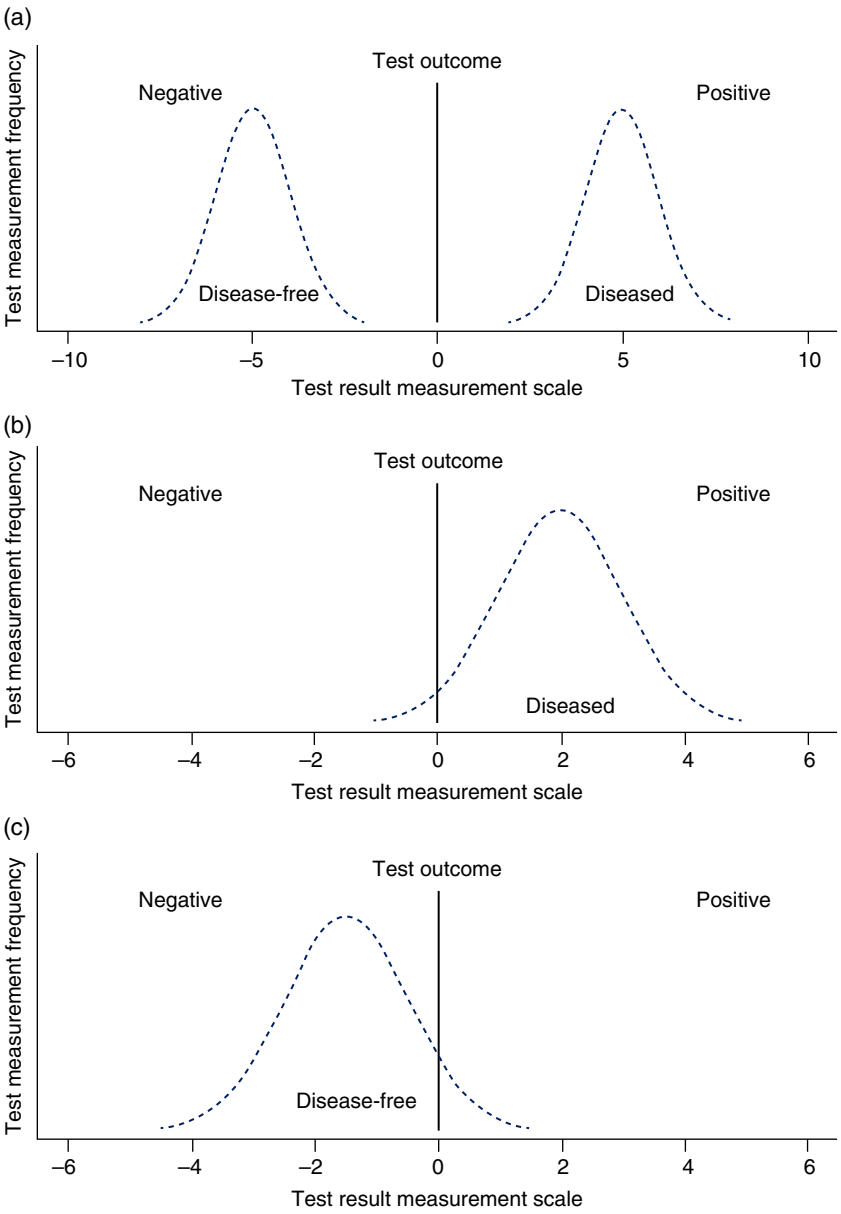


Figure 2.9 Positive and negative test outcome – the ideal and the reality. This is a simplified depiction of the relationship between disease status and test outcome. Given a normal distribution of the measured hormone (which is the exception) and a defined test cut-off (the vertical line), we would like to have positive results (right part of the graph) only in the diseased and negative results (left part of the graph) only in the disease-free individuals (a). The reality is shown below: some of the diseased have a negative test outcome (b) and some of the disease-free have a positive test outcome (c). *Source:* From Matthews and Farewell [21].

mistakes and the complexity of the endocrine system, which cannot be completely reflected by a single hormone test. Dealing with this imperfection requires taking a critical view of the possibilities and limits of hormone tests in use.

The outcome of a test and the true status of a patient can be shown in a 2×2 table (Table 2.2). *Sensitivity* means the probability of an ill individual being detected by the test and *specificity* means the probability of a

Table 2.2 A 2×2 table displaying the possible outcomes of a diagnostic test.

		True disease status	
		Diseased	**Disease-free**
Test outcome	Positive	True positive	False positive
	Negative	False negative	True negative

healthy individual being wrongly detected by the test (Table 2.3). A test with a sensitivity and specificity near 100% is very good but these test characteristics are studied during the initial approval process and estimates are sometimes too optimistic. The possibility of not covering the entire phenotypic spectrum of healthy and ill individuals among those recruited for an approval in an appropriate clinical setting may elicit promises that cannot hold true [20]. For clinical decision-making, it is important to bear in mind that without sensitivity and specificity of 100% any test result can be erroneous.

Table 2.3 The formulas defining the major parameters of test validity.

Parameter	Formula
Diagnostic sensitivity in %	Diseased tested positive/all diseased × 100
Diagnostic specificity in %	Non-diseased tested negative/all non-diseased × 100
Positive predictive value (PPV)	Diseased tested positive/all tested positive
Negative predictive value (NPV)	Non-diseased tested negative/all tested negative
Positive likelihood ratio (LR+)	Percentage of diseased tested positive/ percentage of non-diseased tested positive
Negative likelihood ratio (LR−)	Percentage of diseased tested negative/ percentage of non-diseased tested negative

For the clinician, the predictive value of a test is more important than its sensitivity and specificity. How accurately does a positive or negative test outcome predict disease or the absence of disease? A positive predictive value (PPV) describes the chance of a patient with a positive test actually having the disease, while a negative predictive value (NPV) describes the chance of a patient with a negative test actually not having the disease. These two values are referred to as *post-test probabilities*. The predictive values are not only dependent on the sensitivity and specificity of the test but also depend heavily on the prevalence or pretest probability of the disease in the group of tested individuals (Figure 2.10). Such values therefore combine test accuracy (its intrinsic capability to produce true values) and the quality of the diagnostic view and decision of the physician. A test with high accuracy used only in the absence of the disease (pretest probability 0%) will produce exclusively false positive results and a test with low accuracy used only in the presence of the disease (pretest probability 100%) will produce exclusively true positive results. If the pretest probability of the disease is high, a positive test result is probably correct but, if the pretest probability of the disease is low, a negative test result is probably correct. The post-test probabilities for the contrary interpretation are low because the sum of both post-test probabilities is always 100%.

From a practical standpoint, a test should be used only if its result changes substantially the probability of an endocrine disease. If the diagnosis is secure (pretest probability close to 100%), even a test with high sensitivity

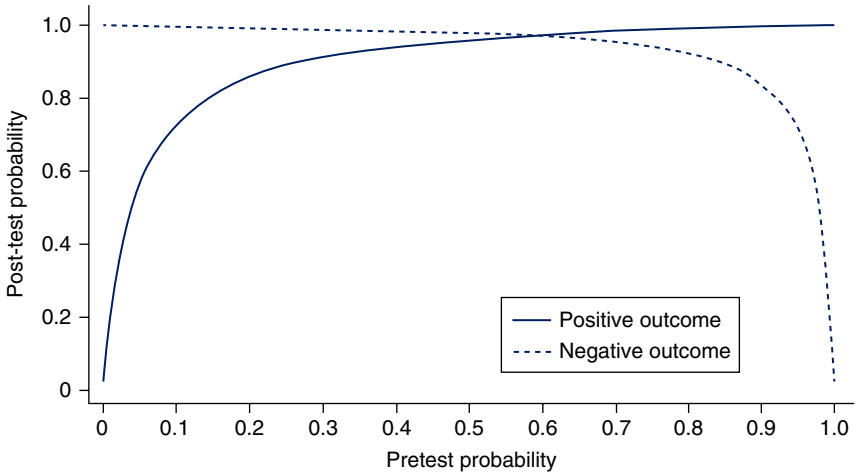


Figure 2.10 Post-test probabilities are dependent on pretest probabilities of the disease. The pretest probability (prevalence of diseased individuals in the tested group) is a major factor in the test prediction: given a diagnostic test with a sensitivity of 98% and a specificity of 96%, the graph shows how the post-test probability that a positive test correctly indicates the presence of disease increases from a very low value when the pretest probability is almost 0 to ~96% when the pretest probability is 50%. This positive predictive value is indicated by the continuous line. In contrast, the post-test probability that a negative test correctly indicates a disease-free state is very high at 98% when the pretest probability is below 50%, but it increases when the pretest probability is within the interval of 50–100%. This negative predictive value is indicated by the broken line. *Source:* Adapted from Matthews and Farewell [21].

and high specificity will not contribute to the diagnosis (Figure 2.10). Conversely, if the pretest probability is very low (<0.1), post-test probability may not differ significantly from pretest probability and the test will not have contributed to the diagnosis (Figure 2.10).

The *positive likelihood ratio* (LR+) is a new term and describes the ratio between the likelihood of obtaining a positive result in the presence of disease and the likelihood of obtaining the same positive result in the absence of disease. In other words, how many more times do diseased individuals show positive results than healthy people? This test characteristic is independent of the pretest probability of disease and is thereby a better quality criterion for an endocrine test than the positive predictive value. A good diagnostic test should have an LR+ significantly above 1. With the knowledge of the pretest likelihood of the disease and the LR+, it is possible to calculate the post-test probability if the test is positive. The negative likelihood ratio (LR–) is defined as the ratio between the likelihood of wrongly obtaining a negative test result in the presence of disease and the likelihood of correctly obtaining a negative result in the presence of health. Therefore, a good test should have an LR– significantly below 1.

As in all quantitative traits, hormone values from a large cohort of human beings, including diseased individuals, have established a continuum from very low to very high values. Therefore, defining a limit that separates disease from health within this continuum is arbitrary and artificial and prone to error. Keeping this in mind is helpful when reading interpretations of laboratory values because it may prevent misjudgement based only on a narrow view of hormone values. Many tests do not make good medical practice.

Using endocrine tests, we can address two different decision limits. The frequently used limits are the 95% confidence interval of hormone concentrations present in healthy individuals but 5% of values outside the 95% confidence interval are also measured in healthy individuals. Thus it is important to emphasize that the probability of the presence of disease correlates with the distance of the hormone value of the patient from these arbitrary decision limits. For instance, a TSH value of 6.5 mU/L makes primary hypothyroidism less likely than a TSH value of 120 mU/L but both are outside the 95% confidence range.

A major problem in pediatric hormone analytics is the scarcity of reference values that require large cohorts of healthy children covering the entire age range from the premature newborn to the adolescent and including the same numbers of individuals from both sexes. For hormones with a circadian rhythm, daytime-dependent reference intervals are also needed. Such examinations in healthy underage children are difficult to obtain for ethical reasons, especially for blood sampling. As a result, most reference values for hormones in blood are made using residual blood after a diagnostic venepuncture for any medical reason. This does not improve the quality of the pediatric reference data reported. A critical look at the numbers of blood samples used for the calculation of pediatric reference data provided by some assay manufacturers shows how unreliable such references are. Numbers well below 50 are unlikely to constitute a reference range from the 2.5th centile to the 97.5th centile; there should be at least 120 individuals for the calculation of these centiles. Hormone reference values are mostly lacking for preterm infants. When no reference data are available, the interpretation of a test result is a guess.

Another possible way to define limits comes from studies comparing test results from patients who were tested because of a similar phenotype and followed longitudinally until the suspected diagnosis could be verified or falsified in each individual [22]. The ideal decision limit (cut-off) is defined at the level with the highest sum of true positives and true negatives. Such an analysis is frequently performed by receiver-operating characteristic (ROC) analysis. This unprejudiced analysis uses each possible result of a diagnostic test as a potential cut-off and calculates its pair of sensitivity and specificity values. The collection of these pairs is plotted on a graph with specificity (false positives) on the x axis and sensitivity (true positives) on the y axis (Figure 2.11). In this trade-off between sensitivity and specificity, the cut-off value that combines the highest number of true test results (positive and negative ones) is used and, in the ROC analysis graph, the point on the curve closest to the upper left corner is usually chosen. The area under the curve (AUC) represents the probability of a correct test result and enables the comparison of the accuracy of different tests for the same diagnostic purpose.

A major problem in measuring hormones is the missing *standardization of immunoassays*. The aim of standardization is to obtain identical and correct results with different assays [23]. GC–MS can be used as a comparator for assaying steroid hormones, catecholamines and thyroid hormones but not for protein hormones where hormone concentrations, such as those of GH and IGF-I, are mostly assay dependent and not directly comparable [24]. Consequently, decision limits for these hormones are assay dependent but this fact is not reflected in endocrine guidelines recommending only one concentration value as a cut-off. In the absence of standardization of assay measurements, endocrine diagnoses are heavily dependent on the laboratory to which the sample was sent. This has been illustrated for the diagnosis of GHD, GH excess and other endocrine diseases, where laboratory values are particularly critical [25, 26].

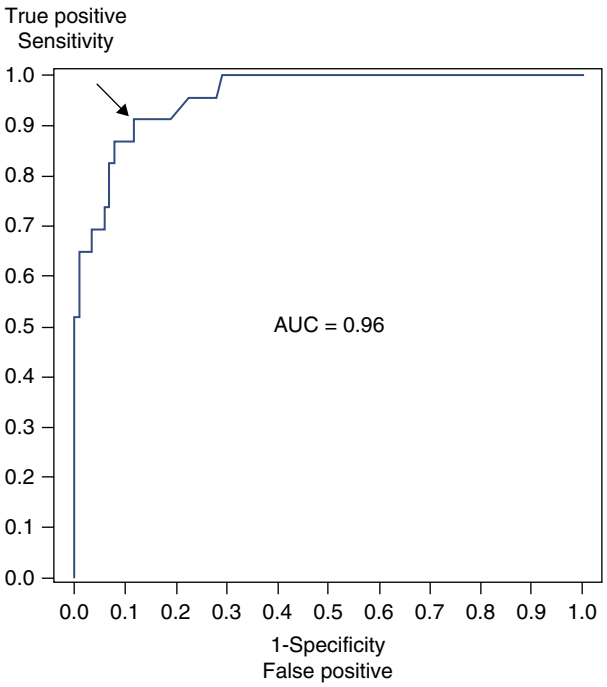


Figure 2.11 Receiver-operating-characteristic (ROC) plot analysis. ROC analysis is a statistical tool for the definition of an unbiased diagnostic cut-off by maximizing both the clinical sensitivity and specificity of a test. The prerequisite of this analysis is the presence of two accurately defined groups comprising diseased individuals and disease-free individuals, ideally with the same group size. Then, for every possible test outcome, sensitivity (positive test in diseased individuals, *Y* axis) and 1-specificity (positive test outcome in disease-free individuals, *X* axis) are plotted on the graph. The test outcome with the highest number of correct results (arrow) is chosen as the cut-off. Here, the cut-off for the GHRH–arginine test for adolescents was determined.

Attempts at decreasing inter-laboratory variability are effective as long as differences in the assays are of a systematic nature but the use of different calibrators is a major problem. The advent of recombinant hormone standards, which are defined by mass units and not by biological activity, was a big step towards standardization and it is now recommended that assay results be reported in mass units and not in biological activity because this property is not measured by immunoassays. The introduction of one single recombinant standard preparation as a calibrator for all GH assays has significantly reduced inter-assay variability. There is also a plea for the exclusive use of monoclonal antibodies that may increase antibody specificity in some immunoassays and reduce lot-to-lot variation since the availability of polyclonal antibodies is always limited.

For basal hormone concentrations, standard deviation scores (SD scores) may have the potential to solve this problem, at least in part. SD scores are age-related standardized concentrations based on a reference cohort. They are defined as the difference between the measured concentration and the mean concentration of the reference cohort divided by the SD of the reference cohort. The SD score interval between −1.88 and +1.88 comprises the values between the 3rd and the 97th centiles when Gaussian distribution is present. In contrast to hormone concentrations, SD scores are ideally assay-independent and enable direct comparison of measurements from different assays but the quality of SD scores depends on the adequacy of the reference cohort (age, gender and puberty related) and on the mathematical model that was used to calculate the SD values, which is frequently ignored.

The same raw data can be converted to different mean and SD values, depending on the use of a parametric or non-parametric approach. Hormone concentration values of a healthy control cohort rarely exhibit a Gaussian distribution but it is necessary to use parametric statistical analysis for the SD calculation. A common solution to this problem is logarithmic transformation of the concentration values since log values frequently follow a Gaussian distribution, allowing the correct calculation of the mean and SD values (Figure 2.12) [3]. Consequently, the correct calculation of an individual SD score of a patient sample concentration first needs a log transformation of the measured concentration. When no mathematical approach results in a normal distribution of raw data, centiles should be used.

In contrast to standardization, *harmonization* aims to provide similar results in different assays but they may all be biased so harmonization is an acceptable goal in the absence of standards. In the Netherlands, for example, GH measurements were harmonized using a central control sample with a defined GH content that was sent to all routine laboratories [26]. This sample was prepared from a serum pool donated by healthy adult individuals after exercise and was initially included in each GH assay run and, after 10 runs, the harmonization factor was calculated by dividing the consensus value by the measured mean value. Afterwards, all GH values measured by the laboratory were corrected according to the harmonization factor. This procedure significantly diminished the inter-laboratory variation in GH measurements in the Netherlands. An alternative approach to harmonization is adjustment of the GH measurement from individual assays by conversion factors or other mathematical methods based on the individual performance of each immunoassay within the relevant concentration interval [27].

Stimulation Tests, Suppression Tests and Profiles

One cornerstone of endocrinology is dynamic testing. Whereas hormone profiles aim to display the endogenous dynamics of spontaneous hormone secretion,

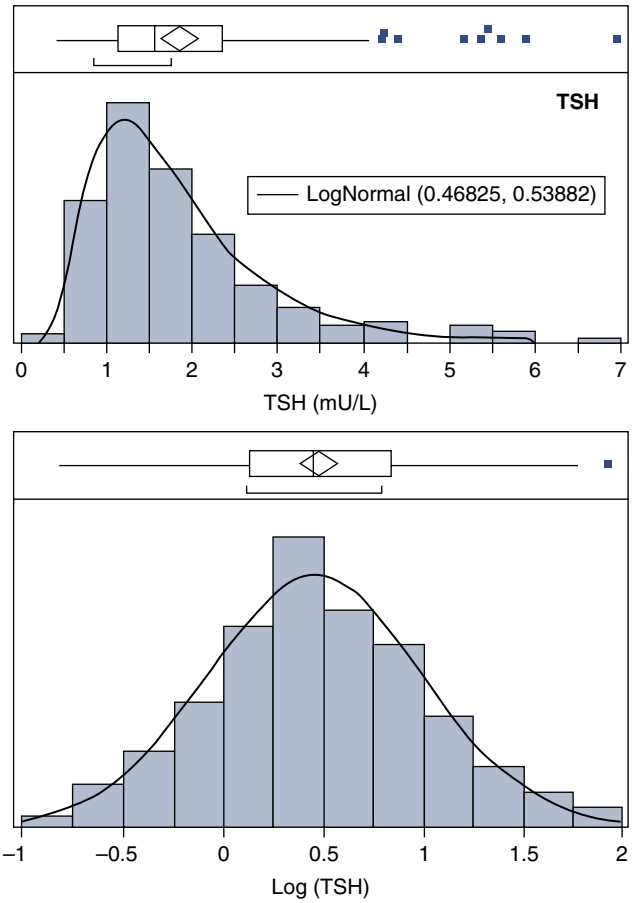


Figure 2.12 Non-parametric distribution of the hormone. The calculation of standard deviation scores (SD scores) requires a Gaussian normal distribution, which is relatively uncommon in endocrinology. The graphs show the frequency of TSH values in a reference cohort. After logarithmic transformation, normal distribution is achieved, and SD scores can be calculated. This mathematical trick is not a cure-all. Therefore, the most recommended reference interval is the central 95% of values (2.5th centile to the 97th centile). *Source:* From Nakamoto and Fuqua [3].

stimulation and suppression tests are used to elucidate the functional state of an endocrine gland or pathway that is not satisfactorily reflected by basal hormone values. These tests are time consuming and may require hospitalization, especially when the tests entrain risk.

It seems plausible that partial hormone deficiencies may be discovered earlier by stimulated rather than by non-stimulated hormone levels [28]. In addition, pulsatile secretion of GH and gonadotropins is not measurable by a single hormone value. Stimulation tests using recombinant hypothalamic or pituitary hormones as a stimulus are powerful challenges that cause the maximum release of a hormone from the target gland in a specific time-dependent manner. Responses to these tests reflect sensitivity to the stimulus and the amount of hormone available over a short time span but they do not necessarily reflect the overall functionality of the gland as is seen in classical GHD compared to neurosecretory dysfunction. Some stimulation tests belong to the standard of endocrine diagnostics, such as the GnRH test for the diagnosis of precocious puberty and GH stimulation tests for the diagnosis of GHD.

The major drawbacks of stimulation tests are their poor standardization and lack of well-established cut-offs [29]. In times of evidence-based medicine and economic restraint in medicine, the use of multiple stimulation tests (e.g. combined pituitary stimulation tests) has faded and been replaced by rational test use when the pretest probability is high enough, the test is sufficiently evaluated and the basal test results are not sufficient to support the diagnosis. For instance, it was shown for the diagnosis of central hypothyroidism that serial T4 measurements are at least as informative as the TRH test result [30]. Only a few suppression tests, such as the dexamethasone suppression test for Cushing syndrome and the glucose suppression test for GH excess, are used.

Profiles best represent hormone secretion but the additional information gained for routine diagnostic purposes is limited. Blood is drawn at specific time intervals or continuously by a pump. There are many different mathematical options on how to extract the message from the raw data: the mean and SD, the AUC (the mean multiplied by the duration of sampling) and the maximum or minimum are available parameters, the most valuable usually being the mean and the maximum peak [29]. More difficult is the identification of the number and amplitude of peaks and several computerized peak detection algorithms, such as PULSAR and Cluster, have been developed. These and other methods are of scientific interest but are not relevant for routine diagnosis of hormone excess or deficiency. Drawbacks for routine use of such profiles are poor reproducibility and high expense.

Table 2.4 Major pre-analytic steps and options.

Step	Choose
Time of appointment	Morning or later; fed or fasting state
Medication	Before or after medication; duration of medication pause
Specimen	Collection tube; immediate cooling; speedy centrifugation
Documentation	Tube labelling and documentation
Intermediate storage	Temperature and time
Shipping	Temperature and timing

Table 2.5 Recall rate, positive predictive value and prevalence of the newborn screening test.

Disorder	Recall (*N*)	Recall (%)	Confirmed cases (*N*)	PPV (%)	Prevalence	Missed patients
Hypothyroidism	528	0.08	207	35.2	1: 3,277	9
CAH	2164	0.33	39	1.3	1: 17,394	2

The number of screened newborns was 678,362. Data are from the screening in Germany in 2010.

Optimal Clinical Use of Hormone Tests

Hormone measurements are ordered by ticking small squares in front of numerous endocrine hormones in laboratory software and sending the probe to a general medical laboratory that covers all measurements of human fluid analytes, including hormones. The patient's laboratory report is frequently accompanied by an automatically derived interpretation validated by the laboratory physician. Although this procedure seems convenient and fast, it does not alter the responsibility of the pediatrician to select the test parameters ticked correctly, the test circumstances, the appropriate use of the collection vials, the correct transport to the laboratory (see pre-analytical steps in Table 2.4) and the clinical evaluation. The frequency of meaningless or erroneous test results can be reduced by the clinician following a few rules [3]:

a) Increase the pretest probability for the disease by restricting tests to individuals with signs and symptoms or those belonging to a high-risk group. In other words, history and clinical examination come first and then the laboratory.

The most prominent, albeit bad, example of this rule is the diagnosis of GHD. GH stimulation tests have a relatively low sensitivity and specificity, with both at 80%. Without priming at the appropriate age, the specificity is likely to decrease to <70% [31, 32]. GHD with a prevalence of 1 in 4000 is much less frequent than idiopathic short stature with a prevalence of 1 in 33 and rarely presents at the age when priming is recommended. Therefore, it seems advisable to limit testing to individuals with sustained low growth velocity, significantly retarded bone age and low IGF-I serum concentrations. These rules significantly increase the pretest probability and dramatically decrease the number of false positives.

There are rare exceptions to this rule and a prime example would be the newborn screening tests for congenital hypothyroidism and congenital adrenal hyperplasia (CAH). The screening test is independent of the clinical phenotype of the patient and the judgement of a physician as it is recommended for every newborn. These tests need high sensitivity for the detection of the disorders and decision limits are defined by specialized laboratories. The non-selective use and the high sensitivity needed cause a high rate of false positive results, as illustrated in Table 2.5. This test weakness is tolerable in these situations where all affected newborns must be detected.

b) Choose the endocrine tests with the highest sensitivity and specificity for the specific situation.

Following this rule may be the most difficult task. This book contains much advice and recommendations of specific endocrine tests for specific disorders but this is only one side of the coin. The other side is the analytical and clinical validity of the specific assay, which we may not know exactly. It is the task of the clinician to obtain the relevant information on the quality of the hormone assays offered by laboratories.

c) Use only those tests the results of which will be important for the management of the patient.

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3

Fetal Endocrinology

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KEY LEARNING POINTS

- The fetal endocrine milieu is vastly independent of maternal hormones due to the placental barrier, which neutralizes the biological activity of hormones during maternal–fetal transfer.
- Complex genetic interactions dictate normal pituitary development. Most cases of congenital hypopituitarism are idiopathic and likely to involve unidentified or multiple genes and/or environmental factors.
- The developing adrenal functions as part of the feto-placental unit. Transcription of steroidogenic genes is tightly regulated in a spatio-temporal manner and the steroids produced have crucial roles in maintaining intrauterine homeostasis and in maturation of the fetus in preparation for extrauterine life.
- The fetal HPA axis is highly susceptible to programming. While endogenous glucocorticoids are inactivated by the placenta, synthetic glucocorticoids readily cross this barrier and should therefore be used only when the benefits clearly outweigh the risks.
- Functionally, the fetus progresses from a state of both primary and tertiary hypothyroidism at mid-gestation, mild tertiary hypothyroidism during the final weeks *in utero*, through to a fully mature hypothalamic–pituitary–thyroid axis by 2 months postnatally.
- Secondary/tertiary hypothyroidism is not diagnosed by TSH-based screening.
- SRY is a critical regulator of male gonadal differentiation. Ovarian development is an equally dynamic process and not a default one; some of the most powerful regulators include the WNT/FZD/CTNNB1, FOXO/FOXL2 and TGF-β/SMAD pathways.
- Male phenotypic differentiation is mediated by testicular testosterone and AMH from the Sertoli cells.
- A multidisciplinary team decision regarding sex of rearing in disorders of sex development is reached after thorough clinical, biochemical and molecular evaluation.
- Catecholamines, produced by sympathetic neurons, neuroendocrine chromaffin cells of the adrenal medulla and extra-adrenal chromaffin cells, are critical for fetal cardiovascular function, response to hypoxia and fetal survival. Labour-induced catecholamine release may aid adaptation to the extrauterine environment.
- Fetal glucose metabolism is largely independent of gluco-regulatory hormones. Fetal energy needs are met by a continuous intravenous supply of glucose across the placenta, with little endogenous glucose production.
- A normal term infant has an immediate postnatal fall in blood glucose concentration during the first few hours of life. Counter-regulatory hormones rapidly become active and these changes result in stabilization of blood glucose concentrations.
- The aetiology of neonatal hypoglycaemia can often be determined from a blood sample taken at the time of hypoglycaemia. Immediate management with glucose correction following investigation can prevent morbidity and mortality.
- Calcium and phosphate are maintained at a higher set point in the fetus than in the pregnant mother, particularly in the third trimester where the demands of fetal bone mineralization are high. The calcium-sensing receptor, present in parathyroid glands, renal tubules, bone and cartilage, and multiple other tissues, plays a

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Companion website: www.wiley.com/go/dattani/brookcpe7

pivotal role in systemic calcium metabolism by modulating production and secretion of PTH, calcitonin, FGF23 and vitamin D and urinary calcium excretion.
- When a neonate presents with an abnormal calcium concentration, assessment of calcium, albumin, phosphate, creatinine, alkaline phosphatase, vitamin D and urine Ca–creatinine ratio and phosphate reabsorption, in both the baby and mother, is important to elucidate the underlying aetiology.
- The regulation of fetal growth is complex involving interactions between genetic, epigenetic and environmental factors. Nutritional substrate transfer via the placenta controls concentrations of IGFs, which are important for fetal growth. Postnatal growth regulators, such as T_4, GH, and sex steroids, play a limited role *in utero* for fetal growth.
- In the fetus anabolism is preserved with negligible hormonal disturbance due to programming of hormonal systems. Catabolic and thermogenic hormone production is restricted. There is minimal variation in substrate supply as the effects of hormones influencing this are limited.
- The fetal adrenal cortex and autonomic nervous system play crucial roles in the immediate transition of the fetus to extrauterine life. Subsequently, maturation of regulatory mechanisms of the PTH–calcitonin system and endocrine pancreas is needed due to discontinuous nutrient supply and transient substrate deficiency.

Introduction

Successful pregnancy resulting in the timely birth of an appropriately mature fetus involves the coordination of the autocrine, paracrine and endocrine networks of maternal–placental–fetal communication. Pregnancy is characterized by sweeping changes in the hormonal profile.

At the beginning of the 20th century, there was recognition that both the fetus and the placenta exert endocrine functions; the classical concept postulated that the placenta acted as a 'temporary endocrine organ' responsible for the steroid biosynthesis evident during gestation. The work of Egon Diczfalusy, who investigated the complex maternal–fetal hormone exchanges that take place and defined the concept of the human feto-placental unit over 50 years ago, was seminal in the field of fetal endocrinology [1].

Placental hormones are secreted in a tightly regulated manner. They are important for establishment of a pregnancy, acting to regulate decidualization, placental development, angiogenesis, endometrial receptivity, embryo implantation and immunotolerance. Following implantation and placentation, the maintenance of pregnancy is crucial to enable fetal development. Subsequently, a diverse range of fetal and neonatal endocrine adaptations enable transition to extrauterine life, which is important as the onset of parturition signals loss of the unique protective *in utero* environment (Table 3.1). A growing understanding of the spectrum of hormones and their mechanisms of action, as well as advances in molecular genetics, have helped to elucidate the physiology of fetal endocrine systems and the pathological basis of many congenital endocrine disorders. Furthermore, hormonal programming during the fetal–perinatal period conditions the functional characteristics of selected adult endocrine systems.

In this chapter we provide an overview of the endocrinology of fetal development, the programming of developing fetal endocrine systems, transition to extrauterine life and advancing frontiers in fetal and neonatal endocrinology. Elucidating the genetic aetiology of a number of congenital endocrine disorders has expanded an evolution of diagnostic and novel therapeutic agents for management of fetal and neonatal endocrine disorders.

Transplacental Passage of Hormones

Growth of the placenta provides an expanding surface area for exchange of substances between maternal and fetal compartments. The chorionic villus forms the basic structural unit of the placenta, comprising vascular projections of fetal tissue surrounded by the outer syncytiotrophoblast, which is in direct contact with maternal blood within the inter-villous space and the inner cytotrophoblast [2]. As the villi mature, there is marked reduction in the cytotrophoblast component so that only a single layer of syncytiotrophoblast separates maternal blood and fetal capillary endothelium at term [2]. The fetal endocrine milieu is largely independent of maternal hormones because the placental barrier is impermeable to most peptides. As the molecular mass of hormones increases, placental transfer decreases, and those that are 0.7–1.2 kD in size have restricted or no access to the fetal compartment [3]. Maternal immunoglobulin G is an exception as this is transported actively by pinocytosis to the fetus in the first trimester, with exponential increase throughout the third trimester [4]. This is important to provide postnatal passive immunity in the first few months of life.

Table 3.1 The fetal endocrine milieu.

Endocrine systems unique to fetal life	
Fetus	*Placenta*
Para-aortic chromaffin system	Production of progesterone
Intermediate pituitary	Ectopic production of pituitary-like hormones (hCG, hCS, or hPL, GH, hCT, ACTH, α-MSH)
Fetal zone of fetal adrenal	Ectopic production of hypothalamic-like neuropeptides (TRH, GnRH, GHRH, CRH, somatostatin)
	Production of growth factors (IGF1, IGF2, VEGF, EGF, FGF, PDGF, TGF)
Feto-placental unit	
Production of oestrogens (oestradiol, oestrone, oestriol, oesterol)	
Prominent fetal hormones or metabolites	
Vasotocin	
Calcitonin	
Cortisone	
Reverse triiodothyronine (r T_3)	
Sulphated iodothyronines	
Neutralization of hormone actions in the fetus	
Receptor or post-receptor immaturity	*Production of inactive metabolites*
T_3	Cortisol inactivated to Cortisone
GH	T_4 converted to rT_3
Prolactin	T_3 converted to T_2
Oestrogens	Catecholamines metabolized by catecholamine-degrading enzymes
	Oestradiol converted to oestrone
Fetal endocrine adaptations	
Fetal adrenal → regulation of placental oestrogen production	
Fetal testis → control of sexual differentiation	

In utero the endocrine environment is distinct, characterized by unique fetal endocrine systems and adaptations, as well as mechanisms to neutralize potent hormone actions to enable survival of a fetus and successful transition to extrauterine life. ACTH, adrenocorticotropic hormone; α-MSH, α-melanocyte-stimulating hormone; CRH, corticotropin-releasing hormone; EGF, epidermal growth factor; FGF, fibroblast growth factor; GH, growth hormone; GHRH, growth hormone-releasing hormone; GnRH, gonadotropin-releasing hormone; hCG, human chorionic gonadotropin; hCS, human chorionic somatomammotropin; hPL, human placental lactogen; hCT, human chorionic thyrotropin; IGF1, IGF2, insulin-like growth factor 1/2; PDGF, platelet-derived growth factor; rT_3, 3,3′5′ (reverse) triiodothyronine; T_2, diiodothyronine; T_3, 3,5,3′-triiodothyronine; T_4, thyroxine; TGF, transforming growth factor; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone; VEGF, vascular endothelial growth factor.

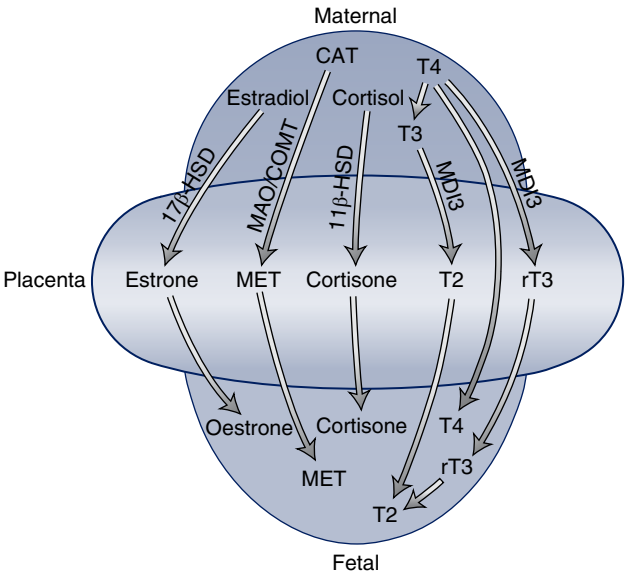

Figure 3.1 Placental neutralization of hormone activity during maternal–fetal transfer. The neutralizing enzymes 17β-hydroxysteroid dehydrogenase (17β-HSD) and 11β-HSD are shown (see text below for details). CAT, catecholamines; COMT, catechol-O-methyltransferase; MAO, monoamine oxidase; MDI3, type 3 iodothyronine monodeiodinase; MET, metamachines; rT_3, 3,3′5′ (reverse) triiodothyronine; T_2, diiodothyronine; T_3, 3,5,3′-triiodothyronine; T_4, thyroxine.

Placental transfer of hormones may involve metabolism and neutralization en route (Table 3.1 and Figure 3.1); examples include cortisol, oestradiol, thyroxine, triiodothyronine and catecholamines (see also 'Neutralization of hormone activity in the fetus') [5–8].

There is almost a 10-fold difference between maternal and fetal cortisol concentrations and such a precipitous gradient could potentially be detrimental were it not for inactivation of maternal cortisol by placental enzymes, which protect the anabolic fetal environment [9]. Placental cells, importantly, contain a type II isoform of 11β-hydroxysteroid dehydrogenase (11β-HSD2) enzyme that converts biologically active maternal cortisol to inactive cortisone [9]. This can be bypassed by administration of dexamethasone to the mother, which leads to fetal exposure to synthetic glucocorticoid. This is used to beneficially promote maturation of the fetal lung in cases of preterm delivery in humans but, although single doses of dexamethasone appear to be safe for mother and infant, more chronic exposure could produce adverse effects [10]. The most recent Cochrane Database review of trials involving over 4730 women and 5650 infants concludes that repeat courses of antenatal glucocorticoids do reduce the risk of respiratory distress syndrome and serious infant outcomes [11] but these benefits were associated with a significant retardation of placental and fetal growth [12]. Low

birth weight is associated with adverse outcomes in later life, such as hypertension, type 2 diabetes, stroke and coronary artery disease. In rodent models, antenatal glucocorticoid exposure has reported adverse effects on blood pressure, blood glucose, hypothalamo–pituitary–adrenal (HPA) axis activity, and memory of the offspring [13, 14]. Furthermore, recent studies have demonstrated initial transgenerational effects in the rat model where *in utero* exposure to glucocorticoids resulted in two generations of offspring exhibiting decreased birth weight and abnormal glucose tolerance compared with controls [15]. These programming effects were transmitted by maternal or paternal lines, implying an epigenetic mechanism. Maternal antenatal corticosteroids are routinely administered to women at risk of preterm delivery with clear benefit to neonatal outcomes. However, use in pregnancy to reduce fetal virilization in a context of congenital adrenal hyperplasia (CAH) still requires careful auditing and research and is not recommended [16, 17].

Before the onset of fetal thyroid hormone (TH) production, the transplacental passage of maternal THs to the fetal circulation in the first trimester is important for fetal neurodevelopment. The human haemochorial placenta regulates the quantity and complement of THs passing through to ensure adequate concentrations are present in the fetus for each stage of development, thus protecting the developing fetus from inappropriately high concentrations of iodothyronines that are associated with fetal loss [18]. Placental tissue contains an iodothyronine inner-ring monodeiodinase, which deiodinates most of the thyroxine (T_4) to inactive reverse triiodothyronine (rT_3) and converts active 3,5,3-triiodothyronine (T_3) to inactive diiodothyronine (T_2) [8, 19]. There is however some transplacental passage of T_4 to the fetus in early pregnancy and significant concentrations of free T_4 (0.5–2 nmol/L) are present in fetal fluids, with low concentrations detectable in the fetal brain from 8 weeks' post-conception (wpc) [20–22]. Infants of mothers with subclinical or mild untreated hypothyroidism are reported to have behavioural and intellectual impairment [23]; however this may also be attributable to transplacental passage of thyroperoxidase (TPO) antibodies [23].

The placenta is the primary site of oestrogen synthesis. Pioneering work by Edward Doisy revealed that three forms of oestrogen (oestradiol, oestriol and oestrone) are present in the urine of pregnant women; these have also been detected in the fetal circulation throughout gestation and in the umbilical cord plasma at birth. By term, oestradiol and oestrone concentrations are 100-fold higher than those of non-pregnant women and oestriol concentrations are 1000-fold higher [24]. Placental 17β-hydroxysteroid dehydrogenase (17β-HSD) coverts active oestradiol to inactive oestrone, thus protecting the maturing fetus from excess oestrogen exposure [7].

Development of Fetal Endocrine Systems

The development of fetal endocrine systems occurs early in gestation in a precise and regulated manner. A central tenet of endocrinology is that circulating concentrations of hormones are tightly controlled: there is a set point around which negative feedback mechanisms maintain concentrations within a range. Pregnancy is a unique state in which fetal and maternal endocrine systems interplay through the transient existence of a third endocrine system, the placenta. Pregnancy progresses ultimately to parturition, when many endocrine axes then elaborate feedforward characteristics. In humans, a dynamic biochemical dialogue exists between fetal endocrine systems, as well as between fetal, maternal and placental units. The complexity of these interactions still remains to be fully elucidated.

Pituitary Development (see also Chapter 5)

Pituitary Embryology

The mature pituitary gland consists of adenohypophysis (anterior and intermediate lobes) and neurohypophysis (posterior lobe). The developing pituitary has a dual embryonic origin: the anterior and intermediate lobes are derived from oral ectoderm, while the posterior pituitary is derived from neural ectoderm, originating from the infundibulum, a specific region of the developing central nervous system (CNS) that forms in the midline of the ventral diencephalon. Although direct evidence is lacking in humans for the processes involved in pituitary development, much information has been derived from other species. These processes appear to be highly conserved across all vertebrate species including zebrafish, amphibians, chick and rodents, and development of the mouse pituitary, in particular, has been well characterized [25–28]. The formation and function of the pituitary depends on the sequential spatiotemporal expression of a cascade of signalling molecules and transcription factors that play crucial roles in organ commitment, cell proliferation, patterning and terminal differentiation. The iterative nature of the inductive interactions required for pituitary morphogenesis makes it very sensitive to both loss-of-function and gain-of-function mutations [29].

The onset of pituitary organogenesis corresponds to 4–6 weeks of gestation in humans. The posterior lobe

comprises axonal projections of neurons, which originate from hypothalamic magnocellular bodies termed the supraoptic, suprachiasmatic and paraventricular nuclei. The former two release arginine vasopressin and the latter releases oxytocin [30]. The anterior pituitary is derived from the pituitary placode. In the mouse, the pituitary placode appears at embryonic day (E) 7.5. Invagination of the placode occurs at E9.0 to form the rudimentary Rathke's pouch, and it is from this that the anterior and intermediate lobes of the adenohypophysis develop. The juxtaposition of Rathke's pouch and the diencephalon is maintained throughout the early phases of pituitary organogenesis, and the tissue interactions between neural and oral ectoderm are critical for the initial stages of pituitary specification.

Development of the Hypothalamus and Pituitary Stalk

The hypothalamus extends from the optic chiasm anteriorly to the mammillary body posteriorly. It is organized into distinct rostral-to-caudal regions, namely, the preoptic, anterior, tuberal and mammillary regions, and three medial-to-lateral regions (periventricular, medial and lateral) [31]. The preoptic nucleus, anterior hypothalamus, dorsomedial nucleus, ventromedial nucleus and the mammillary nuclei are located within the medial region. The preoptic and hypothalamic areas are found in the lateral region [31].

Invagination of Rathke's pouch causes ventral evagination of a portion of the ventral diencephalon forming the infundibulum and later the posterior pituitary and stalk. The pituitary stalk contains the hypophyseal portal system as well as the neuronal connections crossing the hypothalamic median eminence. Within the median eminence itself at the base of the hypothalamus is the capillary bed into which the hypothalamic parvocellular neurons secrete hypophysiotropic hormones. These stimulate the release of the seven anterior/intermediate pituitary hormones via the hypophyseal portal system. The parvocellular neurons also secrete oxytocin and arginine vasopressin, although at much lower concentrations than the magnocellular neurons, with the parvocellular-derived arginine vasopressin acting synergistically with corticotropin-releasing hormone (CRH) in regulating adrenocorticotropic hormone (ACTH) release [30]. It is therefore evident that it is the hypothalamus acting through the pituitary gland, which is the central mediator of growth, reproduction and homeostasis [32].

Studies are slowly elucidating hypothalamic development, which is inherently complex, and perhaps due to its anatomical location and diverse collection of cell groups and neuronal subtypes, there is a paucity of data regarding its regulation at a molecular level [33, 34]. Furthermore, genetic expression studies within the hypothalamus have knock-on effects on multiple neuronal subtypes and downstream physiological processes, making these processes difficult to decipher.

Development of the Human Fetal Hypothalamus and Pituitary

As early as 3 weeks of gestation, the fetal forebrain can be identified, with the diencephalon and telencephalon distinguishable by 5 weeks. Separation of Rathke's pouch from the primitive pharyngeal stomodeum occurs by 5 weeks of gestation [32, 35, 36], and the hypothalamus, pituitary stalk and posterior pituitary are largely developed by 7 weeks. By this time, the floor of the sella turcica is also present, separating the adenohypophysis from the primitive gut. By 10–14 weeks of gestation, hypothalamic neurons containing the neuropeptides somatostatin (SS), growth hormone-releasing hormone (GHRH), thyrotropin-releasing hormone (TRH) and gonadotropin-releasing hormone (GnRH) are evident. Interconnecting fibre tracts are demonstrable by 15–18 weeks of gestation. Maturation of the pituitary portal vascular system continues and becomes functional by 30–35 weeks of gestation when there is portal vascular extension into the hypothalamus.

The definitive Rathke's pouch comprises proliferative progenitors that gradually relocate ventrally. In the developing mouse embryo, a proliferative zone containing Sox2-expressing progenitor cells, capable of giving rise to all cell types within the anterior pituitary, is maintained in a periluminal area and persists in the adult [37–39]. Differentiated anterior pituitary cell types, which include lactotrophs, somatotrophs, corticotrophs, thyrotrophs and gonadotrophs, are present between 7 and 16 weeks of gestation [36]. Secretory granules are evident within anterior pituitary cells by 10–12 weeks and all pituitary hormones can be identified by immunoassay between 10 and 17 weeks' gestation [36, 40].

Genetic Regulation of Pituitary Development

Normal pituitary development is a tightly coordinated process, carefully regulated by a cascade of complex genetic interactions, involving transcription factors and signalling molecules that play critical roles in organ commitment, cell proliferation, cell patterning and terminal differentiation [32] (Chapter 5, Figure 5.4).

Initially, cells within the primordium of the pituitary gland are competent to differentiate into all cell types. Sequential expression of signalling molecules and transcription factors at critical periods of pituitary development then occurs and is subsequently attenuated (Figure 3.2). Following expression of the earliest markers of pituitary gland development, such as homeobox gene

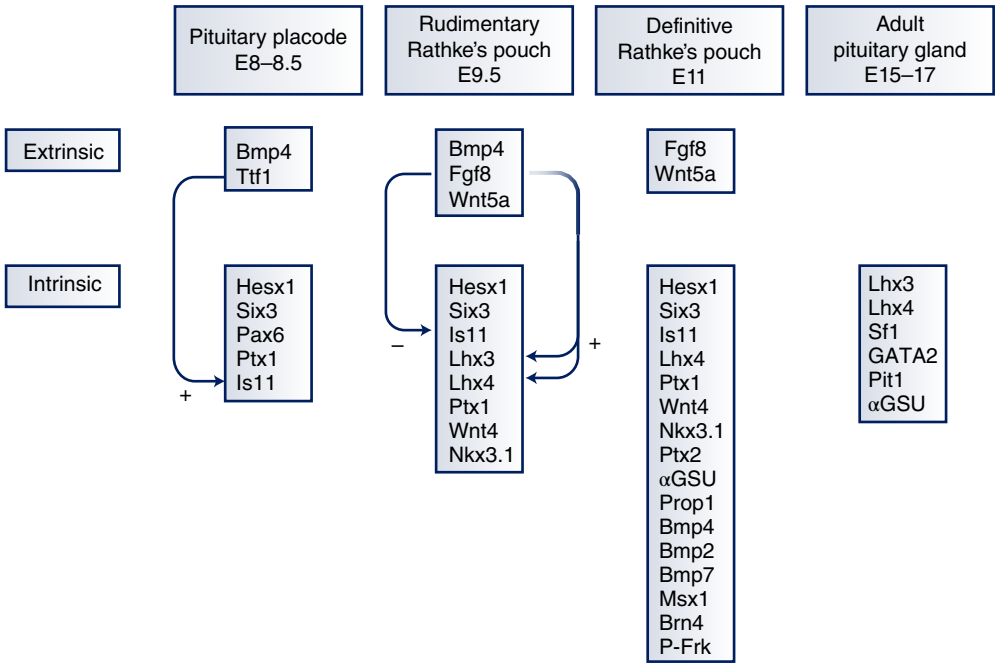

Figure 3.2 Transcription factors and signalling molecules involved in anterior pituitary development.

expressed in embryonic stem cells (*Hesx1*), further signalling pathways are established that direct these cells towards terminal differentiation. Genes expressed early are not only implicated in organ commitment but are also implicated in repression and activation of downstream target genes that have specific roles in directing the cells towards a particular fate.

Significant insights into human pituitary disease have been gleaned from spontaneous or artificially induced mutations in the mouse. Identification of mutations associated with human pituitary disease has, in turn, been invaluable in defining the genetic cascade responsible for the embryologic development of this vital endocrine organ. Mutations involved specifically in human hypothalamo–pituitary disease are detailed in Table 3.2 [41].

A number of genes are implicated in syndromic forms of hypothalamo–pituitary disease:

GLI3 – Plays a role in sonic hedgehog signalling; haploinsufficiency results in Pallister–Hall syndrome associated with polydactyly, hypothalamic disorganization, hypothalamic hamartoma and hypopituitarism [42, 43].

PITX2 – A mutation in this gene has been identified as one cause of Axenfeld–Rieger syndrome involving ocular, dental and hypothalamic abnormalities [44]. *Pitx2* knockout mice have pituitary hypoplasia and decreased *Ghrh receptor*, *Gh*, *Fsh*, *Lh* and *Tsh* gene expression [44].

ARNT2 – A basic helix-loop-helix transcription factor critical for normal development of the paraventricular and supraoptic nuclei. Mutations in this gene are associated with severe pituitary insufficiency, including growth hormone (GH), TSH and ACTH deficiencies, and diabetes insipidus (DI) with progressive microcephaly, seizures, severe visual impairment, severe learning difficulties and abnormalities of the renal and urinary tracts [45].

Rare isolated pituitary hormone deficiencies have been associated with mutations in the respective hypothalamic releasing hormones or the releasing hormone receptors, e.g. familial GH deficiency due to *GHRHR* mutations, TSH deficiency due to *TRHR* mutations and gonadotropin deficiency due to *GNRHR* mutations [46–48]. No genetic aetiology has been identified in most cases of congenital hypopituitarism, suggesting a role for other unidentified genes or environmental factors.

Fetal Pituitary Production of GH and PRL

Human GH and PRL are both produced from the somatotroph cells within the anterior pituitary. The fetal pituitary gland can synthesize and secrete GH by 8–10 weeks of gestation. Pituitary GH content increases from about 1 nmol (20 ng) at 10 weeks to 45 nmol (1000 ng) at 16 weeks of gestation. Fetal plasma GH concentrations are in the range of 1–4 nmol/L during the first trimester and increase to approximately 6 nmol/L in mid-gestation. Plasma GH concentrations fall progressively during the second half of gestation to a mean concentration of 1.5 nmol/L at term [36]. Human somatotrophs respond predominantly to GHRH at 9–16 weeks, while response to the inhibitory SS develops later in gestation, leading to a progressive fall of plasma GH during the second half of

Table 3.2 Comparison of murine and human phenotypes in hypothalamo-pituitary development [41].

Gene	Protein	Murine loss-of-function phenotype	Human phenotype	Inheritance (murine and human)
HESX1	HESX1	Anophthalmia or microphthalmia, agenesis of corpus callosum, absence of septum pellucidum, pituitary dysgenesis or aplasia	Variable: SOD, CPHD, IGHD with EPP. Anterior pituitary hypoplastic or absent. Posterior pituitary ectopic or eutopic. Frequency of mutations: <1%	Dominant or recessive in humans, recessive in mouse
OTX2	OTX2	Lack of forebrain and midbrain, olfactory placode, optic placodes	Anophthalmia, APH, ectopic posterior pituitary, absent infundibulumFrequency of mutations: 2–3% of anophthalmia/microphthalmia cases	Heterozygous: haploinsufficiency/dominant negative
SOX2	SOX2	Homozygous null mutants: embryonic lethal. Heterozygous mice and further dose reduction: poor growth, reduced fertility, CNS abnormalities, anophthalmia; pituitary hypoplasia with reduction in all cell types	Hypogonadotropic hypogonadism; APH, abnormal hippocampi, bilateral anophthalmia/microphthalmia, abnormal corpus callosum, learning difficulties, oesophageal atresia, sensorineural hearing loss, hypothalamic hamartoma. Frequency of mutations: 8/235	*De novo* haploinsufficiency in humans, heterozygous mutation associated with haploinsufficiency in mouse
SOX3	SOX3	Poor growth, weakness, craniofacial abnormalities, ACC, hypothalamic and infundibular abnormalities	IGHD and mental retardation, hypopituitarism; APH, infundibular hypoplasia, EPP, midline abnormalities. Frequency of mutations: 6% (duplications), 1.5% (mutations)	X-linked recessive in both mice and humans
GLI2	GLI2	N/A	Holoprosencephaly, hypopituitarism, craniofacial abnormalities, polydactyly, single nares, single central incisor, partial ACC. Frequency of mutations: 1.5%	Haploinsufficiency in humans
FGF8	FGF8	Holoprosencephaly, reduction of vasopressin and oxytocin, reduced GnRH neurons	Holoprosencephaly, hypogonadotropic hypogonadism, ACTH and TSH deficiencies, diabetes insipidus	AR in both human and mouse, AD in some cases
LHX3	LHX3	Hypoplasia of Rathke's pouch	GH, TSH, gonadotropin deficiency with pituitary hypoplasia. ACTH insufficiency variable. Short, rigid cervical spine. Variable sensorineural hearing loss.Frequency of mutations: 1.3%	Recessive in both
LHX4	LHX4	Mild hypoplasia of anterior pituitary	GH, TSH, cortisol deficiency, persistent craniopharyngeal canal and abnormal cerebellar tonsils; APH, ectopic/eutopic posterior pituitary, absent infundibulum. Frequency of mutations: 1.2%	Recessive in mouse, recessive/dominant in humans
PROP1	PROP1	Hypoplasia of anterior pituitary with reduced somatotrophs, lactotrophs, thyrotrophs, corticotrophs and gonadotrophs	GH, TSH, PRL and gonadotropin deficiency. Evolving ACTH deficiency. Enlarged pituitary with later involution. Frequency of mutations: 1.1% sporadic cases, 29.5% familial cases	Recessive in both
POU1F1	POU1F1 (PIT1)	Anterior pituitary hypoplasia with reduced somatotrophs, lactotrophs and thyrotrophs	Variable anterior pituitary hypoplasia with GH, TSH and PRL deficiencies. Frequency of mutations: 3.8% sporadic cases, 18% familial cases	Recessive in mouse, dominant/recessive in humans
ARNT2	ARNT2	Anterior pituitary hypoplasia, TRH, somatostatin, oxytocin and CRH deficiencies, reduced vasopressin neurons	TSH, GH, ACTH deficiencies, DI, small anterior pituitary, vesicoureteric reflux, renal impairment, visual impairment, neonatal seizures with progressive microcephaly	Autosomal recessive
PNPLA6	PNPLA6	N/A	Oliver–McFarlane syndrome; trichomegaly, congenital hypopituitarism and retinal degeneration with choroidal atrophy; hypogonadotropic hypogonadism	Autosomal recessive
TCF7L1	TCFL1	Forebrain, eye and pituitary deficits	Septo-optic dysplasia with variable hypopituitarism	Autosomal dominant

Source: Reproduced with permission of Oxford University Press.
ACC, agenesis of corpus callosum; ACTH, adrenocorticotropic hormone; APH, anterior pituitary hypoplasia; CNS, central nervous system; CPHD, combined pituitary hormone deficiencies; EPP, ectopic posterior pituitary; GH, growth hormone; IGHD, isolated growth hormone deficiency; N/A, not applicable; PRL, prolactin; SOD, septo-optic dysplasia; TSH, thyroid-stimulating hormone.

gestation to 1.5 nmol/L at term [36]. The responses of plasma GH to SS and GHRH and to insulin and arginine are mature at term in human infants [36, 49]. The high plasma GH concentrations after development of the pituitary portal vascular system at mid-gestation may reflect unrestrained secretion [36]. GH secretion is already pulsatile soon after birth in humans [50] but trough concentrations are still higher than in later life so that random GH sampling may be used to detect GH deficiency in the neonatal period, which is not possible at a later age.

Concentrations of fetal plasma PRL, in contrast to GH, are low until 25–30 weeks' gestation and increase to an average peak concentration of approximately 11 nmol/L at term (Figure 3.3) [51–53]. Pituitary PRL content increases progressively from 12 to 15 weeks and PRL release increases in response to TRH and decreases in response to dopamine. The hypothalamic control of PRL matures only late in gestation and during the first months of extrauterine life [36, 49]. The marked increase in fetal plasma PRL concentration in the last trimester parallels the increase in fetal plasma oestrogen concentrations, although it lags by several weeks [36, 49].

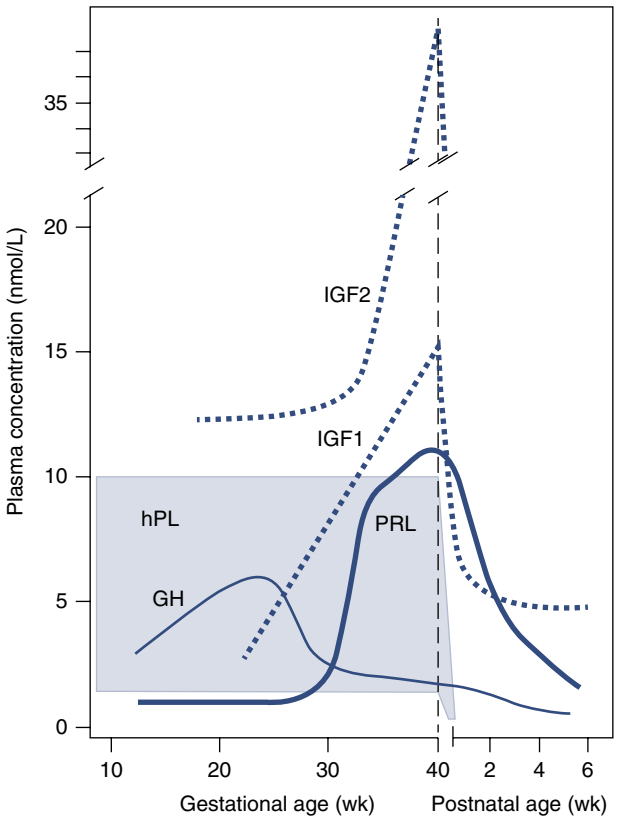

Figure 3.3 Patterns of change of fetal plasma human placental lactogen (hPL), growth hormone (GH), prolactin (PRL), insulin-like growth factor 1 (IGF1) and insulin-like growth factor 2 (IGF2) during gestation and in the neonatal period. The shaded area indicates the range of fetal plasma hPL concentrations. *Source:* Data from Bala et al. [51], Bennett et al. [52], and Kaplan et al. [53].

Postnatally, GH acts through receptors in liver and other tissues to stimulate production of insulin-like growth factor 1 (IGF1) and, to a lesser degree, insulin-like growth factor 2 (IGF2). Antenatally, however *GH receptor* mRNA levels and receptor binding are low in fetal liver, although receptor mRNA is present in other fetal tissues [36]. The growth of anencephalic fetuses, and initially of neonates with congenital GH deficiency, is almost normal, suggesting that factors other than GH stimulate fetal insulin-like growth factor (IGF) production with nutrition probably playing the most important role [54, 55]. PRL receptors are present in most fetal tissues during the first trimester of gestation and it is likely that lactogenic hormones have a significant role in organ and tissue development early in gestation [40, 55]. In particular PRL appears to promote fetal growth and skeletal/adipose tissue maturation and might also stimulate lung surfactant synthesis, as cord serum concentrations of PRL are reduced in infants who develop respiratory distress syndrome.

Placental lactogens (PL), also known as chorionic somatomammotropins (CSH), are encoded for by three genes: *PL-A*, *PL-B* and *PL-L*. These are located on chromosome 17q22, which contains a growth hormone/placental lactogen (*GH/PL*) gene cluster, including two related genes that encode pituitary GH (*GH-N*) and placental GH (*GH-V*). Placental GH is produced in the syncytiotrophoblast [56]. It rises sharply after mid-gestation to a peak at 34–37 weeks and, within 1 hour after delivery of the placenta, it disappears from the circulation [57]. Placental GH is secreted into the maternal circulation and reduces insulin sensitivity in the mother and so spares glucose and other nutrients for fetal growth.

PL is structurally homologous to GH but functionally closer to PRL and is secreted directly into both fetal and maternal circulations. PL is detectable in the maternal circulation at 6 weeks of gestation and fetal PL concentrations are approximately 20–50 ng/mL at term. Lactogens affect insulin production, and at least in rodents, evidence suggests that PRL and PL signalling through the PRL receptor is essential for the increase in pancreatic β-cell mass [58]. PL also affect hypothalamic gene expression and leptin action in the mother and so maintain metabolic homeostasis while providing the substrates for nutrition for the fetus and neonate.

Fetal Production of Ectopic Hypothalamic-like and Pituitary-like Hormones

ACTH-like immunoreactivity is present in relatively high concentrations in the neonatal rat pancreas and kidney [59], presumably derived from a pro-opiomelanocortin (POMC) parent molecule as hypothalamic neuropeptides

are present in fetal gut and tissues originating from it. POMC is secreted by the neuroendocrine cells of fetal lung and may be important as part of a stress response or for the timing of parturition by altering adrenal sensitivity to circulating ACTH. These cells also produce vasoactive intestinal peptide (VIP) and serotonin.

High concentrations of TRH and SS immunoreactivity have been reported in neonatal rat pancreas and gastrointestinal tract tissues [60, 61]. Hypothalamic concentrations of TRH are low and while encephalectomy does not alter circulating TRH levels in the neonatal rat, pancreatectomy results in a significant reduction. In the sheep fetus, THs modulate pancreatic and gut TRH concentrations, pointing to TH control of extrahypothalamic TRH in the fetus [62]. TRH and SS are also present in the human neonatal pancreas and in blood of the human newborn, with both hormones derived mostly from extrahypothalamic sources [63–66]. These data suggest a role for extrahypothalamic TRH in the control of fetal pituitary TSH secretion before the near-term maturation of hypothalamic TRH. The role of extraneural SS in the fetus remains unclear. Ghrelin is more abundant in the fetal pancreas (in contrast to postnatal expression, which is mostly in stomach and hypothalamus) and is also present in fetal lung although its function here remains to be clarified [67, 68].

Human chorionic gonadotropin (hCG) is produced by human fetal kidney, liver and testes from 16 to 20 weeks of gestation and is bioactive *in vitro* [59, 69]. The hCG/LH receptor is present in fetal organs including the kidney, liver, lung, spleen and intestine and hCG may promote organ growth and differentiation but no clear function has been demonstrated to date [70].

Adrenal Development

Adrenal Embryology

The adrenal cortex develops from a thickening of the intermediate mesoderm known as the gonadal ridge, in contrast to the adrenal medulla, which derives from the ectoderm. The adrenal gland appears as the bipotential adrenogonadal primordium (AGP) at 28–30 days' post-conception (dpc) in humans [71] due to expression of the transcription factor steroidogenic factor-1 (*SF-1*) (*NR5A1*), a nuclear receptor essential for adrenal development and steroidogenesis (Figure 3.4).

Gonadal cells migrate caudally. Cells destined to generate the adrenal cortex express the highest levels of *NR5A1* and migrate to the cranial pole of the mesonephros to form the adrenal primordium (AP) at 33 dpc. Migration of neural crest-derived cells to the AP occurs around 48 dpc and these cells are located in scattered clusters throughout the fetal adrenal. After birth these neural crest-derived cells coalesce, differentiating into the medullary chromaffin cells of the adrenal. Following neural crest invasion, encapsulation of the AP occurs by 7 wpc, resulting in the formation of a distinct organ just above the developing kidney.

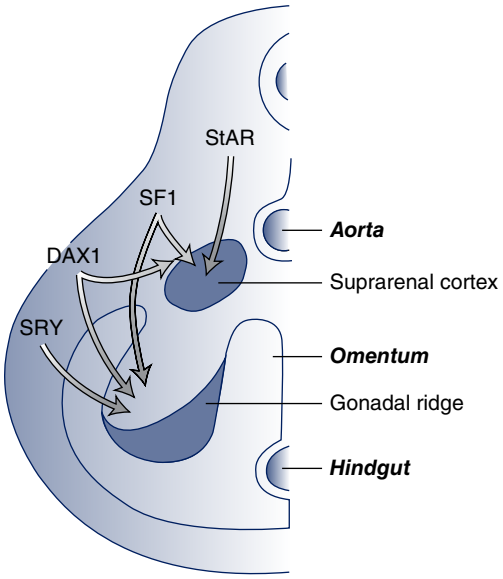

Figure 3.4 Hemi cross section of a 5-week human embryo showing the locations of the adrenal primordia (suprarenal cortices) and gonadal ridges. Steroidogenic factor 1 (SF-1), NR5A1 is involved in testicular and ovarian development. SRY is the single critical regulator of testicular embryogenesis. Inactivation of the DAX1 (dosage-sensitive sex reversal, adrenal hypoplasia congenital [AHC], X chromosome factor), NR0B1 gene leads to adrenal hypoplasia. The steroidogenic acute regulatory protein (StAR) is the rate-limiting factor for adrenal steroidogenesis (see text for details).

Two distinct zones of the developing adrenal cortex form by 50–52 dpc. The sizeable inner cortical zone, referred to as the fetal zone (FZ), contains large eosinophilic-rich polyhedral cells, which express high levels of steroidogenic enzymes. The narrow outer cortical zone, referred to as the definitive zone (DZ), is composed of small tightly packed basophilic-rich cells, expressing much lower levels of steroidogenic enzymes. A third zone, the transition zone (TZ), can be identified from 14 wpc. The TZ is located between the DZ and FZ and contains cells with histological appearances of both [72, 73].

Following encapsulation, the fetal adrenal (FA) grows rapidly, undergoing tremendous change as pregnancy progresses largely due to an increase in size of the FZ, which accounts for 80–90% of the mass of the gland by mid-gestation [74, 75]. Growth of the human fetal adrenal (HFA) is most rapid from the second trimester and by 18 weeks the gland is approaching the size of the kidney. The fastest growth occurs during the last 6 weeks of gestation, mainly due to an enlarging FZ. The FA forms 0.4% of body weight at term, weighing 3–5g with a relative size 10- to 20-fold that of the adult adrenal [72].

The main function of the FA during pregnancy is steroidogenesis, specifically increasing C19 androgen production with brief key periods of increased cortisol secretion. These steroids maintain intrauterine homeostasis and prepare the fetus for extrauterine life.

FZ cells strongly express the enzyme cytochrome P450 17 alpha (CYP17), which has both 17-hydroxylase and 17,20-lyase activities. CYP17 coverts pregnenolone to dehydroepiandrosterone (DHEA). DHEA is sulphated to dehydroepiandrosterone sulphate (DHEA-S) and this acts as a substrate for placental aromatization [72]. FZ cells also produce other sulphated Δ5 steroids, including pregnenolone sulphate and 17α-hydroxypregnenolone sulphate but their functional role is unclear [71, 72].

Remodelling of the adrenal cortex occurs during the neonatal period, although this process is not well understood. FZ involution is reported to occur immediately after birth due to apoptosis of cells, with a parallel decrease in adrenal androgen production [71]. A 50% reduction in weight of the adrenal gland is reported to occur within the first 2 weeks' post-partum [76]. However, whether the timing of FZ involution is determined by birth or gestation remains controversial. FZ androgen production has been reported to persist in infants born prematurely [77] and involution of the FZ may therefore be linked to maturation rather than the event of birth itself.

In contrast, a more recent study has demonstrated a similar pattern of rapid adrenal involution with size of the adrenal gland, as measured by ultrasonography, decreasing to its normal infantile size within the first 2 weeks after birth in all neonates examined, regardless of their gestational age at birth [72]. The FZ is absent by 6 months of age in most cases.

Adrenal cells with zona reticularis (ZR) morphology are seen from about 3 years of age until a continuous ZR forms at around 6 years of age. At this stage adrenal androgen synthesis recommences, a phase referred to as adrenarche [78]. The exact mechanisms for ZR growth and the factors regulating adrenarche remain elusive. The adult cortex develops from the DZ and TZ, giving rise to the zona glomerulosa (ZG) and zona fasciculata (ZF), respectively.

Genetic Regulation of Adrenal Development

Several genes and growth factors regulate fetal adrenocortical development and human and animal models of disordered development have provided insights into underlying mechanisms (see Chapter 9).

The temporal expression of key genes is critical in the control of adrenal development. Those regulating formation of the intermediate mesoderm and urogenital ridge affect kidney, adrenal and gonadal development; genes governing AGP development affect both adrenal and gonadal development; there are also those genes that affect only adrenal development specifically (Table 3.3).

The genes encoding *NR5A1* and *DAX-1* (dosage-sensitive sex reversal, adrenal hypoplasia congenital [AHC], X-chromosome factor, *NR0B1*), an orphan member of the nuclear receptor superfamily, have emerged as pivotal in the regulation of AGP development and are expressed in adrenal cortex, testis, ovary, hypothalamus and pituitary [73]. In the absence of *NR5A1* expression, the adrenal gland does not form [71]. Severe disruption of *NR5A1* in humans can cause adrenal dysfunction, although most pathogenic variants in *NR5A1* in humans cause impaired testicular development and Leydig cell dysfunction rather than adrenal insufficiency. Overexpression of *Nr5a1* in mice causes atypical proliferation and neoplasia in the mouse adrenal, while *Nr5A1* haploinsufficiency results in delayed/incomplete adrenal development. In the developing adrenal, data indicate that while Wilms' tumour 1 (*WT1*) regulates *NR5A1* expression in the AGP, Cbp/P300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain 2 (*CITED2*) expression in the AGP is necessary for differentiation of the AP and FZ [71].

The presence of a fetal adrenal-specific enhancer (FAdE) in the Nr5a1 locus that directs transgene expression to the fetal adrenal cortex was identified by Zubair et al. [79]. It was demonstrated that this enhancer is autoregulated by *Nr5a1* and acts as a critical regulator of *Nr5a1* gene expression in the mouse AP. The transcription complex, which contains the homeobox protein PKNOX1 (*Prep1*), homeobox gene 9b (*Hox9b*) and pre-B-cell leukaemia transcription factor 1 (*Pbx1*), initiates FAdE-mediated *Nr5a1* expression in the AGP. *Nr5a1* subsequently regulates itself by maintaining FAdE-mediated *Nr5a1* expression in the AP. After E14.5 in mice, FAdE is no longer utilized. In the emerging DZ, *Nr5a1* regulation is shifted to a different definitive enhancer that has not yet been characterized. In humans, however, no similar FAdE or DZ enhancers have yet been confirmed [71].

In the rodent *Nr5a1* up-regulates *Nr0b1*, which in turn represses *Nr5a1* transcriptional activity and, hence, steroidogenesis. It is unclear how disruption of *Nr5a1* and its negative regulator can cause similar defects. *Nr0b1* can function as a co-activator for *Nr5a1* transcriptional activity in steroidogenic cells when expressed at high levels and the observations *in vivo* may be complicated by the pleiotropic effects of these factors.

Pathogenic variants or deletions of *NR0B1* in humans are well established as the cause of X-linked AHC [80]. Knockdown of *Nr0b1* results in premature differentiation of mouse adrenocortical progenitor cells. However, this occurs at the expense of depleting this essential cell

Table 3.3 Comparison of murine and human phenotypes in adrenal development.

Gene	Protein	Murine loss-of-function phenotype	Human phenotype
Development of the urogenital ridge			
Odd1	Odd1	*Odd1*-null mutants: embryonically lethal. No condensation of metanephric mesenchyme, impaired urogenital ridge formation. Complete absence of adrenal glands, gonads and kidneys in those that survive to 15.5 dpc	
WT1	WT1	*Wt1*-null mutants: embryonically lethal. Kidney development does not extend beyond thickening of the coelomic epithelium Mutants partially rescued by a human WT1 transgene survive to birth with urogenital abnormalities and much impaired adrenal development	Germline mutations cause defects in gonad and kidney formation
Sall1	Sall1	*Sall1*-null mutants: severe kidney dysgenesis or agenesis, hypoplastic adrenal glands at birth Murine model that phenocopies the deletion seen in Townes–Brocks syndrome; absent adrenal glands and kidneys and hypoplastic gonads at 16 dpc	Townes–Brocks syndrome (heterozygous mutations); renal and genital abnormalities
Pbx1	Pbx1	*Pbx1* knockout: embryonically lethal with adrenal agenesis, impaired testis development *Pbx1*$^{+/-}$ mutant: smaller adrenal glands with a ZF containing fewer, hypertrophied, cells and fewer proliferative cells in the subcapsular region	
Wnt4	Wnt4	*Wnt4*-null mutant: impaired kidney development from 15 dpc defects of the female reproductive system, Sertoli cell differentiation in the testes and adrenal glands with reduced CYP11B2 expression and Pref-1/Dlk1 expression	Homozygous missense mutation in *WNT4* shown to be associated with renal agenesis, gonadal defects and adrenal hypoplasia from 19 weeks' gestation
FoxD1 *FoxD2*	FoxD1 FoxD2	Homozygous null *FoxD1* and *FoxD2* mutants: hypoplastic adrenal glands at birth as well as developmental defects of the kidneys and ureter	
Development of the adrenogonadal primordium			
NR5A1	SF-1	*Nr5a1* gene knockout mutant: adrenal and gonadal agenesis, gonadotropin deficiency and absence of the hypothalamic ventromedial nucleus	Heterozygous loss-of-function mutations – 46,XY and XX DSD, complete gonadal dysgenesis, primary adrenal failure No cases of homozygous mutations have been reported to date
DAX-1	Dax1	Adrenal hypoplasia Gonadotropin deficiency	AHC – adrenal insufficiency of both glucocorticoids and mineralocorticoids, with an adrenal cortex that has poorly developed adult zones but retention of cytomegalic FZ cells
IGFR1	IGFR1	A mouse model in which both the insulin receptor and Igf1r are constitutively deleted; reduced growth, gonadal dysplasia with male-to-female sex reversal and adrenal developmental defects ranging from severe hypoplasia to agenesis	
Development of the adrenal primordium			
Wnt/β-Catenin	β-Catenin	The β-catenin-null adrenal: smaller than wild-type adrenal gland at 14.5 dpc and contains many fewer *Sf-1*-positive cells By 18.5 dpc, the gland is no longer detectable	
Cited2	Cited2	Deletion of *Cited2*: embryonically lethal with mutants exhibiting adrenal agenesis by 17.5 dpc	

Odd1, odd-skipped-related 1; WT1, Wilms' tumour 1; SF-1, steroidogenic factor 1; DAX1, dosage-sensitive sex reversal, adrenal hypoplasia congenital (AHC), X chromosome factor; IGFR1, IGF1 receptor; Cited2, CBP/p300-interacting transactivator with ED-rich tail.

population, ultimately resulting in adrenal failure. As such, *Nr0b1* plays an essential role in the maintenance of stem/progenitor cell pluripotency. *Nr5a1* activates *Nr0b1* transcription in cooperation with paracrine Wnt signalling and glucocorticoids that are synthesized in the differentiated adult cortex. Conversely, ACTH, the well-established glucocorticoid stimulator, has been shown to effect release of *Nr5a1* complexes from the *Nr0b1* promoter, thus leading to effective inhibition of *Nr0b1* transcription [81, 82]. This is predicted to promote the response of Nr5a1-positive progenitor cells to ACTH and subsequently initiate steroidogenesis.

Adrenocortical Growth and Zonation

The exact origin of adrenocortical cells remains debatable. It is postulated that undifferentiated pluripotent stem cells exist in the adrenal cortex to maintain homeostasis in the adult. Enucleation studies in rodents, together with dye-labelling observations, suggest a capsular origin for these stem cells. Proliferation studies and lineage tracing experiments in mice suggest that progenitor stem cells are located in subcapsular/DZ pools [79] and that these differentiate as they move centripetally through the gland between zones [73]. An alternative theory is that a population of stem cells exists in the FZ/TZ and these migrate bidirectionally to populate the DZ and expand the FZ. Other studies focussing on a downstream activator of the hedgehog pathway, glioma-associated oncogene homologue 1 (zinc finger protein) (Gli1), suggest that it is the adrenal capsular cells that give rise to the DZ. Gli1-expressing cells, located in the adrenal capsule, do not express Sf-1. King et al. demonstrated that this subpopulation of cells is capable of giving rise to Sf-1-expressing differentiated adrenocortical cells during embryonic development [83]. More recent evidence suggests that a combination of these theories may be correct, with the FZ contributing to a subscapular progenitor cell population that then differentiates through the zones to maintain stable cell populations in the developed mature adrenal [73]. Wood et al. showed that FZ cells that once expressed Sf-1 under control of the FAdE enhancer gave rise to a subset of capsular cells. These FZ cell descendants within the adrenal capsule express Gli1, suggesting some FZ cells can change into Sf-1-negative capsular cells, which in turn can give rise to the underlying DZ/adult adrenal cortex [84].

Some conditions associated with adrenal hypoplasia (for example, IMAGe syndrome, X-linked AHC) may reflect a defect in progenitor cell expansion during early fetal development.

Signalling Pathways in Adrenal Development

Sonic hedgehog (Shh) is a member of the vertebrate hedgehog (Hh) family of secreted ligands and performs a multitude of crucial roles during embryonic development. It is also required in the adult for tissue maintenance, differentiation and the regulation of stem cell populations. Secreted Hh ligands bind to the 12-pass transmembrane protein Patched-1 (Ptch1), which relieves the inhibition it exerts on the G-protein-coupled receptor smoothened (Smo) and allows it to prevent the processing of the Gli transcription factors. Full-length Gli3 and Gli2 act as transcriptional activators. Gli1, which only acts as a transcriptional activator, is not expressed in the absence of Hh but is up-regulated by the pathway. Shh signalling is required for normal adrenal development at a later stage than *NR5A1* and *DAX1*. In the mouse Shh is expressed in the developing adrenal in cells just beneath the capsule [83, 85, 86]. Shh expression marks cortical progenitors [85] and Shh-expressing cells are capable of giving rise to all steroidogenic cells in the cortical zones. Homozygous deletion of *Shh* in murine embryos is lethal. Analysis of the adrenal gland at 14.5 and 16.5 dpc in *Shh*-null embryos, however, indicates that the AP does form, but is much smaller than in the wild type [83, 86].

Holoprosencephaly is a consequence of the inactivation of the Hh pathway, with defects observed in SHH [87], PTCH1 and GLI2, and is often associated with adrenal hypoplasia [88]. Smith–Lemli–Opitz syndrome (SLOS), a condition due to a mutation in 7-dehydrocholesterol reductase required for the formation of cholesterol, is associated with abnormalities that overlap those seen in cases of impaired Hh signalling, including holoprosencephaly [89]. SLOS is also associated with adrenal insufficiency [90], which may be due to the requirement of cholesterol as the substrate for steroidogenesis or may be because Hhs are covalently linked to cholesterol [91], which is critical for their signalling, or because 7-dehydrocholesterol, which accumulates in the plasma of SLOS patients, can be transported out of the cell by Ptch1 and acts as a negative regulator of Smo [92].

Fibroblast growth factor (FGF) signalling has been shown to be an important regulator of a number of early developmental processes, such as anterior/posterior patterning and organogenesis [93]. FGFs are a large family of secreted glycoproteins that bind to four FGF receptors, FGFR1–FGFR4. Embryos with a global *Fgfr2b* deletion have hypoplastic adrenal glands [94] and deletion of both isoforms of *Fgfr2* from steroidogenic tissue recapitulates this phenotype and causes male-to-female sex reversal, implying that Fgfr2 is not necessary for AGP formation but is required for the subsequent growth and development of the adrenal gland [95].

Epidermal growth factor (EGF) stimulates proliferation of both the FZ and the DZ. The fetal adrenal expresses high levels of *IGF2* mRNA and protein, which

are responsive to ACTH [96]. IGF2 augments ACTH-stimulated expression of steroidogenic enzymes and stimulates steroid hormone production in fetal adrenal cortical cells. The pattern of enzyme maturation in the fetal adrenal suggests that cortisol production does not occur *de novo* from cholesterol until 30 weeks' gestation but some production using progesterone as precursor probably occurs earlier [96].

CDKN1C (cyclin-dependent kinase inhibitor 1 C, *P57KIP2*) is a paternally imprinted gene located on chromosome 11p.15, encoding the CDKN1C protein, which inhibits cell cycle progression [73]. Variations in *CDKN1C* or its genomic imprinting can lead to adrenal pathology. Loss of function of *CDKN1C* results in Beckwith–Wiedemann syndrome (BWS), an overgrowth syndrome with increased susceptibility to adrenal carcinoma. IMAGe (intrauterine growth restriction [IUGR], metaphyseal dysplasia, AHC and genital anomalies) syndrome is a rare multisystem disorder [97] that mirrors the features of BWS and is caused by gain-of-function mutations in *CDKN1C*. The most clinically important component of IMAGe syndrome is adrenal insufficiency, comprising loss of both mineralocorticoid and glucocorticoid synthesis, which can be life threatening shortly after birth [97].

Fetal Adrenal Steroidogenesis

The fetal adrenal expresses the same five steroidogenic apoenzymes as the adult adrenal [72]: *CYP17A1* (P450c17, 17-hydroxylase/17,20-lyase), *CYP21A2* (P450c21, 21-hydroxylase), *CYP11A1* (P450scc, side-chain cleavage) and *CYP11B1/CYP11B2* (P450c11/aldosterone synthase). A fifth enzyme, expressed by the smooth endoplasmic reticulum (ER), exhibits both 3β-HSD and Δ^4,Δ^5-isomerase activities.

Transcription of steroidogenic genes is tightly regulated in a spatio-temporal manner, resulting in differences in the zonal activity of these enzymes [72].

The principal output from the fetal adrenal during pregnancy is DHEA from the FZ. The DZ/TZ contributes only a small fraction of total fetal adrenal steroid production. DHEA is sulphated in the FZ to DHEA-S, which is then converted by placental aromatase to oestrogens. The human placenta cannot synthesize oestrogens *de novo* and aromatization accounts for 50% of the oestrone and oestradiol and 90% of the oestriol in the maternal circulation [72].

Cortisol is produced by the TZ, with an early peak from 8 to 9 wpc coinciding with transient expression of type 2 3β-hydroxysteroid dehydrogenase (*HSD3B2*) [98]. The HPA axis is sensitive to glucocorticoid-mediated feedback at this time; 46,XX female fetuses with steroidogenic defects (for example, in CYP21 or CYP11) lack cortisol and have an elevated ACTH drive. This stimulates excess production of fetal androgens, at a time when the genital folds are sensitive to such exposure. This can result in virilization of female external genitalia [98]. Cortisol can slow fetal and placental growth and so is converted to biologically inactive cortisone by the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2) in placental and fetal tissues. In this way the fetus is protected from high concentrations of cortisol *in utero*. Concentrations of circulating cortisone in the fetus at mid-gestation are 4–5-fold higher than cortisol concentrations (Figure 3.5) [99–101].

As term approaches, expression and activity of 11βHSD2 decreases and selected fetal tissues including liver and lung express 11βHSD1. This ensures maternal glucocorticoids are available to the fetus and act to drive maturation of key fetal organs. The increase in fetal cortisol production has an important role for neonatal survival and adaptation to extrauterine life (see 'Fetal adaptations for transition to extrauterine life'). In the sheep the number of glucocorticoid receptors (GR) in the fetal hypothalamus increases at term, suggesting that some process in the fetus allows the normal autoregulation of GR to be overridden at term [102]. Mice lacking GR function demonstrate an enlarged and disorganized

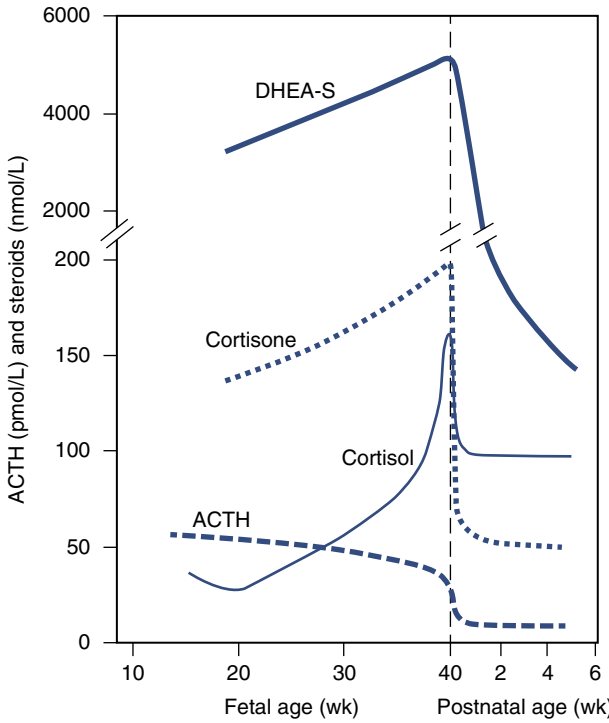

Figure 3.5 Patterns of change of fetal plasma adrenocorticotropic hormone (ACTH), cortisol, cortisone and dehydroepiandrosterone sulphate (DHEA-S) during gestation and in the neonatal period. The trend of average values is shown for each hormone in nanomoles per litre. Notice the broken scale for DHEA-S. *Source:* Data from Winters et al. [99], Murphy [100], and Winter [101].

adrenal cortex, atrophy of the adrenal medulla, lung hypoplasia and defective gluconeogenesis [71]; they do not survive for long after birth.

Premature delivery is physiologically a unique event. The very act of existing is inherently stressful for a preterm infant, who may also be less likely to mount a stress response and exhibit signs of adrenocortical insufficiency, particularly in the first week of life. After birth, biologically active cortisol predominates over cortisone in the circulation. Healthy term infants have initial pulsatility of cortisol secretion and can develop a circadian rhythm from as early as 1 month post-partum, although there is considerable variability. There is, however, no consensus about the definition of a 'normal' cortisol concentration in preterm infants and these may indeed vary greatly depending on gestational age at birth. Studies suggest that shortly after birth cortisol concentrations decline to a nadir over approximately 8 weeks and remain at a low level for at least several additional weeks [103]. Antenatal steroids for a threatened preterm delivery have resulted in a clear reduction in mortality and morbidity. However, unlike endogenous glucocorticoids that are inactivated by 11βHSD2 in the placenta, synthetic glucocorticoids readily cross the placenta. In the fetal sheep, hypothalamic and pituitary GR are present at mid-gestation and corticotropin suppressibility can be demonstrated by the midpoint of the third trimester of gestation [102]. There may well be consequences of such early exposure to supraphysiological steroid concentrations, as the HPA axis is highly susceptible to programming during development and the full impact of long-term effects are as yet unknown. Longitudinal follow-up of the offspring of women treated with betamethasone for threatened preterm birth has shown that individuals exhibit insulin resistance 30 years after treatment, particularly women. Previously, antenatal treatment of the fetus of a mother whose previous child has CAH by administering dexamethasone orally to the mother at 8–12 wpc was condoned. This was found to reduce fetal androgens and hence the virilization of female fetuses. However due to clear lack of high quality evidence in terms of efficacy and safety, this should be regarded as a highly controversial experimental treatment [17]. The constant dexamethasone dose used can result in cortisol concentrations that exceed physiological fetal concentrations by around 60-fold. To date, a number of side effects and adverse associations have been reported, including neurological abnormalities in the offspring [17].

Use of steroids postnatally in preterm infants to facilitate extubation can reduce the risk of chronic lung disease. This treatment however is associated with hyperglycaemia, hypertension, gastrointestinal bleeding and perforation, hypertrophic cardiomyopathy, growth failure and cerebral palsy and is not recommended [104]. Early low-dose hydrocortisone treatment has been reported to improve survival in very preterm infants without bronchopulmonary dysplasia. In an analysis of secondary outcomes of the PREMILOC randomized clinical trial (early low-dose hydrocortisone to improve survival without bronchopulmonary dysplasia in extremely preterm infants), early low-dose hydrocortisone was not associated with a statistically significant difference in neurodevelopmental outcomes at age 2 years [105]. Trials involving more physiological doses of hydrocortisone are currently underway, but all systemic and inhaled steroids are associated with adrenal suppression in neonates, and further longitudinal studies are required.

The HFA gland is capable of aldosterone secretion near term with functional development of the DZ. Aldosterone regulates renal sodium excretion in fetal sheep and in premature infants [106]. Fetal plasma aldosterone concentrations in infants who are born by caesarean section are reported to be 3–4-fold higher than maternal concentrations [101]. Vaginal delivery and maternal salt restriction increase aldosterone concentrations in both mother and infant. The mechanism for high aldosterone concentrations in the fetal and neonatal periods remains unclear. The increased fetal aldosterone concentrations are thought to be due to increased fetal adrenal secretion and these persist during the first year of extrauterine life. Mineralocorticoid receptors (MRs) are present in fetal tissues from 10 to 14 wpc [107]. MR immunoreactivity is detectable in a wide range of fetal tissues including kidney, skin, hair follicles, trachea and bronchioles, oesophagus, stomach, small intestine, colon and pancreatic exocrine ducts, although the role of MRs in these tissues remains unclear. *MR* knockout mice appear normal at birth but demonstrate defects in mineralocorticoid function and the renin–angiotensin system (RAS) in the postnatal period [108].

Angiotensin II concentrations in the sheep fetus are similar to maternal concentrations and blockade of fetal production with angiotensin-converting enzyme (ACE) inhibitors decreases the fetal glomerular filtration rate (GFR) [109]. Both subtypes of angiotensin receptors, AT_1 and AT_2, are detectable in various tissues, including kidney, early in fetal development [108]. Hormonal factors modulate fetal renal *AT* gene expression in sheep: angiotensin II suppresses both AT_1 and AT_2 and cortisol increases AT_1 gene expression in kidney and lungs [110]. There is poor correlation between plasma renin activity (PRA) and aldosterone concentrations in cord blood [106]. The role of the RAS in fetal development is not clear; rather than modulating renal sodium excretion through aldosterone, it may maintain renal excretion of salt and water into amniotic fluid to prevent oligohydramnios [109].

Manifestations of mineralocorticoid deficiency in the newborn term infant can occur because of aldosterone deficiency or competition for binding to renal MRs by other steroids such as 17-hydroxyprogesterone (17-OHP). A relatively reduced neonatal GFR limits sodium loss initially, but by 1 week of age aldosterone deficiency results in the characteristic manifestations of hyponatraemia, hyperkalaemia and volume depletion.

Endocrine Regulation of Adrenal Development

ACTH, a 39-amino acid peptide secreted from the anterior pituitary under control of CRH, is a major stimulus for fetal adrenal function. ACTH binds to the transmembrane receptor melanocortin receptor 2 (MC2R) in adrenocortical cells and exerts its effect through downstream signalling pathways. The growth-promoting effects of ACTH are mediated partly via stimulation of locally produced growth factors such as IGF2 and fibroblast growth factor β (FGFβ) [71]. While adrenocortical growth and differentiation appear to be independent of ACTH during the first trimester of human pregnancy, ACTH begins to play an important role in the morphological and functional development of the adrenal after 13 wpc [111].

In vivo ACTH activates the enzyme StAR, increasing delivery of cholesterol to P450scc and stimulating steroidogenesis [101], and excess ACTH is clearly involved in driving the high androgen concentrations seen in fetuses affected with CAH. *In vitro* ACTH has been shown to directly stimulate DHEA-S and cortisol production. The HPA axis clearly functions early in fetal life but anencephalic fetuses, which lack pituitary ACTH, have adrenals that contain a fairly normal complement of steroidogenic enzymes and retain their capacity for steroidogenesis [73]. Furthermore, the pattern of growth and escalating steroid production by the fetal adrenal during pregnancy do not appear to be paralleled by increasing concentrations of fetal plasma ACTH. There may be gestational age-dependent alterations in responsiveness of the fetal adrenal tissue to ACTH; however it appears both ACTH-dependent and ACTH-independent mechanisms regulate fetal adrenal development.

The presence of all CRH peptides and their receptors in the human adrenal suggests that this system may be crucial for adrenal growth and development. Placental CRH is an important source, releasing CRH into the fetal circulation during human pregnancy. As gestation advances, the concentration of CRH concentration increases. The rapid rise in placental CRH production at the end of gestation has been suggested to contribute to the process of parturition by forming a feedforward loop that leads to increased production of cortisol and DHEA/DHEA-S by the fetal adrenal. *In vitro* CRH stimulates cortisol production in primary cultures of fetal adrenocortical cells by increasing expression of mRNA levels of *StAR* (steroidogenic acute regulatory protein) and other steroidogenic enzymes, such as *HSD3B2*, *CYP21A2* and *CYP11B1*. In addition, CRH enhances the adrenal response to ACTH, further driving the production of cortisol and DHEA/DHEA-S [112]. *CRH* gene knockout in mice results in neonatal death due to pulmonary hypoplasia, suggesting that CRH-stimulated glucocorticoid production is essential for adrenergic chromaffin and normal lung development.

The circulating concentrations of CRH increase 1000-fold as pregnancy progresses [113] and reach concentrations of 0.5–1 nmol/L at term; normal concentrations in non-pregnant women are <0.01 nmol/L. At the end of pregnancy, there is increased bioavailability of CRH due to a fall in concentrations of its binding protein. This results in an exponential increase in maternal CRH concentrations from 35 weeks' gestation to term [113, 114]. Post-partum, CRH normalizes to non-pregnant values within 24 hours of delivery, in keeping with the fact that the placenta is the primary source of CRH during gestation [113, 114]. Placental CRH is bioactive and concentrations correlate with maternal cortisol concentrations, suggesting that circulating placental CRH plays a role in stimulating maternal corticotropin release. Mid-gestation, fetal plasma ACTH concentrations average approximately 55 pmol/L (250 pg/mL), concentrations that maximally stimulate fetal adrenal steroidogenesis, and concentrations are higher throughout gestation than in postnatal life, although they fall near term [59, 101] (Figure 3.5).

The Feto-placental Unit

The paradox of HFA function is that steroidogenesis is programmed largely to produce inactive products [101]. The gland is programmed by the steroidogenic enzyme expression pattern (for example, relative 3β-HSD deficiency) to produce inactive DHEA and pregnenolone and their sulphate conjugates, even though it is maximally stimulated to maintain fetal cortisol concentrations and ACTH feedback homeostasis. Much of the DHEA is converted to 16-hydroxy-DHEA by the fetal adrenal and fetal liver. This is designed to provide DHEA substrate for placental oestrone and oestradiol production. 16-Hydroxy-DHEA undergoes metabolism to oestriol in the placenta. Fetal DHEA-S production and maternal oestriol concentrations increase progressively as term approaches and DHEA-S production can reach as much as approximately 200 mg/day [59]. Suppression of placental oestrogen in pregnant baboons by an aromatase inhibitor increases the volume of the FZ markedly [115]. This effect can be reversed by administration of inhibitor plus oestrogen, suggesting that oestrogen selectively suppresses FZ growth and development

during the second half of gestation in the primate. This is thought to represent a feedback mechanism to regulate secretion of fetal adrenal DHEA, thereby maintaining normal fetal–placental function [115].

Neonatal Adrenal Insufficiency

Adrenal insufficiency presenting in the neonatal period is a rare condition that may occur secondary to ACTH deficiency or is primarily resulting from adrenal failure. This condition is covered in detail in Chapter 9, but the practical approach to a neonate presenting with adrenal insufficiency is briefly considered below (Figure 3.6). A diagnosis of adrenal insufficiency must be prompt in view of the potential life-threatening consequences of glucocorticoid and mineralocorticoid deficiencies to the infant.

Thyroid Development

Thyroid Embryogenesis

Two anlagen contribute to the developing thyroid, one of the first endocrine organs to develop. The precursor of the T_4-producing follicular cells is formed by 22 dpc from a midline thickening of the pharyngeal floor, known as the median anlage. The parafollicular calcitonin-secreting (C) cells derive from paired ultimobranchial bodies originating from the fourth pharyngobranchial pouches, known as the lateral anlagen [22].

Between 28 and 48 dpc thyroid precursor cells undergo caudal migration, proliferation and lateral expansion. This whole process is known as lobulation. Fusion of the lateral and median anlagen occurs at about 44 dpc. A persisting epithelial stalk, the thyroglossal duct, connects the thyroid to the pharynx during descent [116]. The thyroglossal duct inserts into the buccal cavity floor, at a point later known as the foramen caecum on the developing tongue. During normal development, the thyroglossal duct has usually disappeared by 37 dpc, leaving behind only the foramen caecum remnant [116]. If the thyroid descent is abnormal, this may result in an ectopic thyroid gland or a persistent thyroglossal duct.

By 51 dpc the gland has formed into two lateral lobes with a connecting isthmus and has descended below the thyroid cartilage, its final position, by 9 wpc. The fetal thyroid weighs about 80 mg at 10 wpc, increasing to 1–1.5 g by term. Once migration is concluded, the process of terminal differentiation begins at around 60 dpc. During differentiation, the expression of genes encoding the TSH receptor (*TSHR*), sodium-iodide symporter (*NIS*), thyroglobulin (*Tg*) and thyroperoxidase (*TPO*) results in thyroid follicles forming with functional capacity [117]. Iodine is needed as a substrate for production of THs, following stimulation of the thyroid by TSH. By 70 dpc, colloid is visible histologically and the thyroid expresses *SLC5A5*, which encodes for the 13-transmembrane domain glycoprotein NIS, enabling uptake of iodine by the thyroid and TH biosynthesis. THs can be detected in the thyroid from 9 wpc and in fetal blood from 10 wpc [118–121].

Thyroid Hormone Production

Pituitary and plasma TSH concentrations begin to increase during the second trimester in the human fetus, at about the time that pituitary portal vascular continuity develops (Figure 3.7) [122–124].

In the rat, significant up-regulation of *Tshr* gene expression is seen on fetal day 17, and this is accompanied by considerable growth and rapid thyroid development in terms of structure and function. Increased expression of Tg and TPO is evident and thyroid follicles capable of thyroid hormonogenesis are seen, suggesting the TSHR plays an important role in these processes. Mutations of *Tshr* in the rat are associated with a phenotype consisting of severe hypothyroidism and a hypoplastic, but normally located, thyroid gland with a poorly developed follicular structure. A similar phenotype in humans is demonstrated in infants of mothers with potent TSHR-blocking antibodies and in babies with severe loss-of-function mutations in *TSHR*.

As pregnancy advances through the last half of gestation, plasma TSH concentrations rise progressively. Plasma concentrations of T_4-binding globulin and total T_4 increase from 14 to 16 wpc to maximal concentrations at term. As total T_4 production rises, free T_4 concentrations also increase. With advancing gestation there is progressive maturation of the hypothalamo–pituitary–thyroid (HPT) axis, particularly during the third trimester, with increasing responsiveness of the thyroid to pituitary TSH resulting in a rise in plasma T_4. Pituitary TSH secretion is responsive to hypothyroxinaemia and to TRH early in the third trimester [119]. The rise in fetal TSH and free T_4 concentrations during the latter half of pregnancy is followed by sequential surges of TSH and free T_4 in the early neonatal period. Subsequently there is a period of equilibration of the TSH/free T_4 ratio to adult values during infancy and childhood [122, 125–127]. During this period there is maturation of hypothalamic TRH secretion, sensitivity of the pituitary to TRH, TSH negative feedback control, and thyroid follicular cell responsiveness to TSH. Production of ectopic TRH from the placenta and fetal pancreas, and decreased TRH breakdown in the circulation, results in higher fetal serum TRH concentrations compared with maternal concentrations. Functionally, the fetus progresses from a state of both primary and tertiary hypothyroidism at mid-gestation through a state of mild tertiary hypothyroidism during the final weeks *in utero* to a fully mature HPT axis by 2 months of postnatal life.

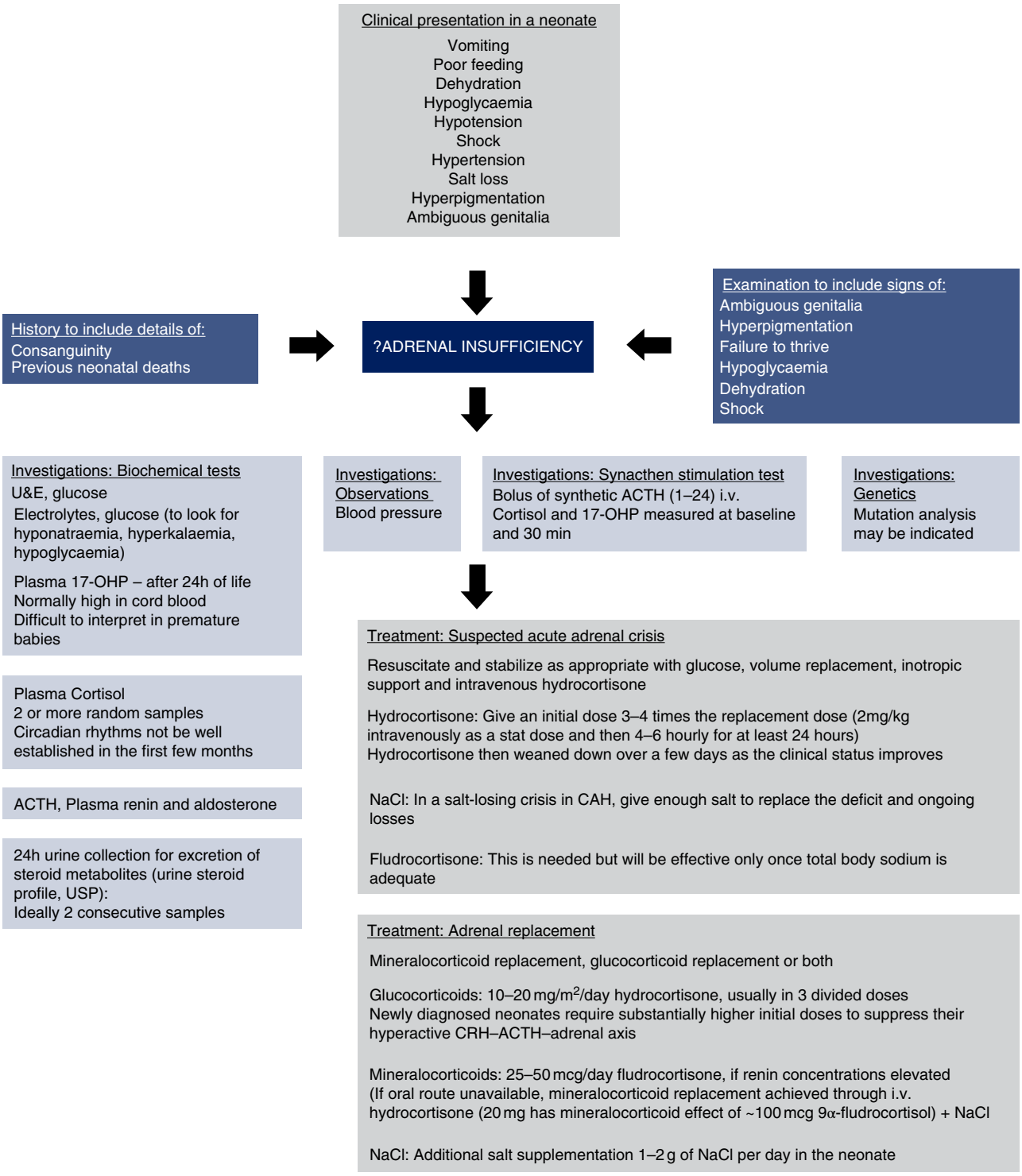

Figure 3.6 Approach to a neonate presenting with signs and symptoms of adrenal insufficiency.

Genetic Regulation of Thyroid Development

At least five developmental genes are involved in thyroid and parathyroid gland embryogenesis (see Chapter 8). These include the genes for thyroid transcription factors *PAX8* (paired box 8), *NKX2-1* (NK2 homeobox 1, previously known as TTF1), *HHEX* (haemopoetically expressed homeobox), *FOXE1* (forkhead box E1, previously known as *TTF2*) and *NKX2-5* (NK2 homeobox 5) (Figure 3.8) [121, 128, 129].

In addition to mediating the formation of the thyroid bud, these transcription factors drive functional

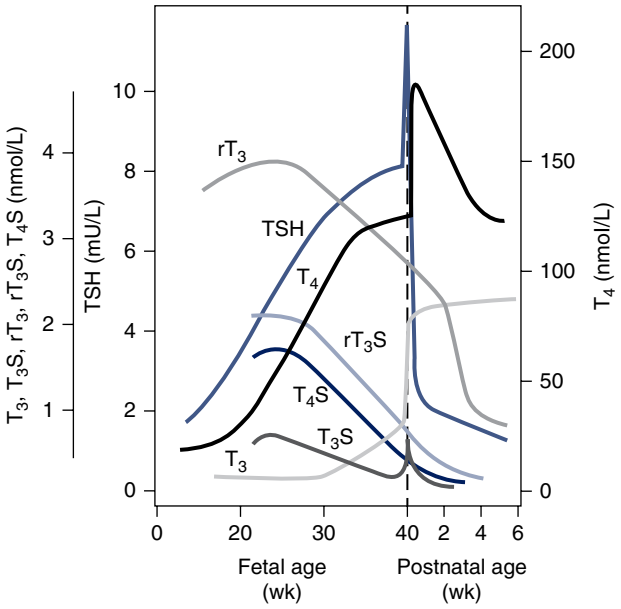

Figure 3.7 Patterns of change of fetal plasma thyroid-stimulating hormone (TSH), thyroxine (T_4), triiodothyronine (T_3), reverse T_3 (rT_3) and iodothyronine sulphates (T_4S, rT_3S and T_3S) during gestation and in the neonatal period. The patterns for T_4S and rT_3S are based on limited 30-week data. Source: Data from Burrow et al. [122], Santini et al. [123], and Fisher and Klein [124].

differentiation, regulate the expression of genes involved in TH biosynthesis and play a role in the maintenance of the mature thyroid gland (see Chapter 8). Mutations in *NKX2-1*, *PAX8* and *FOXE1* are all associated with thyroid dysgenesis in humans [119, 121, 130–132].

Signalling Pathways in Thyroid Development

Evidence from mouse and zebrafish studies suggests that thyroid morphogenesis is guided by cues from extrinsic factors such as permissive signals from blood vessels [133]. Genes known to contribute to thyroid morphogenesis in animal models include the Notch ligand *Jag1*, the thyroid and cardiac transcription factor *Nkx2-5*, and *Ntn1*, which is implicated in zebrafish aortic arch artery formation and thyroid morphogenesis [134–137]. Other autonomous factors that may play a role in thyroid morphogenesis include components of the FGF signalling pathway, *Tbx1*, a transcriptional regulator of the FGF signalling pathway [120], and regulators of apoptosis [138].

The Shh signalling pathway has also been implicated in murine thyroid development [133]. Shh appears to have an important role in the patterning and process of symmetrical bilobulation late in organogenesis, as well as suppressing ectopic expression of thyroid follicular cells [139].

Thyroid Function in Preterm Infants

Thyroid function in the preterm infant reflects an immaturity of the HPT axis, which is related inversely to gestational age [127]. When compared with term infants, in the preterm neonatal free T_4 increments are reduced in infants born at 31–34 weeks' gestation, attenuated at 28–30 weeks and absent at 23–27 weeks [140]. Age-specific changes in thyroid function in children with SGA and normal birth weight are available [141]. The premature delivery of an infant terminates the changes in TH metabolism that occur during the third trimester. This results in low T_4 and T_3 concentrations that increase in parallel with increasing gestational age at delivery in preterm infants [140]. TH concentrations appear to be even lower than those expected *in utero* [142] due to loss of maternal free T_4 in association with an immature HPT axis, low iodide stores and reduced iodothyronine deiodinase 1 (DIO1) enzyme activity.

A natural postnatal nadir in thyroxine concentration occurs at 7 days of life with a subsequent rise thereafter

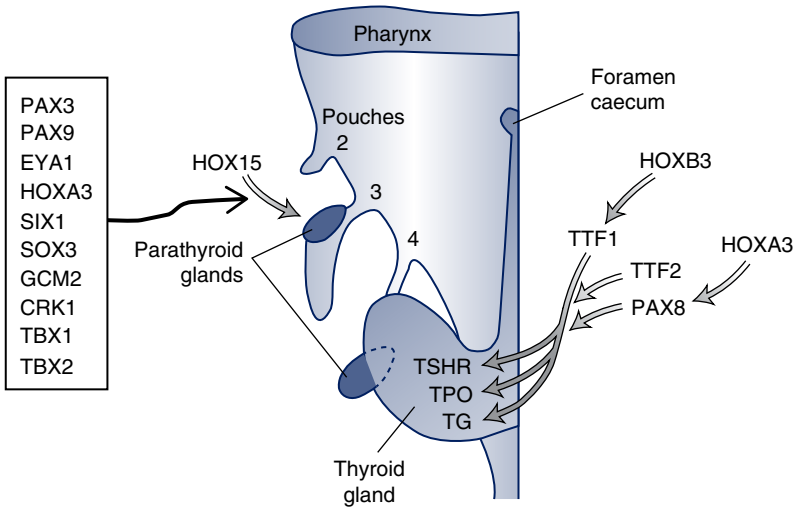

Figure 3.8 Illustration of the homeobox genes that program development of the thyroid and parathyroid glands. *HHEX* is involved early in the integrated cascade that programs thyroid gland embryogenesis. *HOXB3* and *HOXA3* may be responsible for activation of thyroid transcription factors *NKX2-1* and *FOXE1*, respectively, during early embryogenesis. *PAX8* is essential in the cascade. These factors are also involved in thyroid follicular cell function, promoting thyroglobulin (*TG*), thyroid peroxidase (*TPO*) and thyroid-stimulating hormone receptor (*TSHR*) gene transcription. *HOX15* gene knockout in mice causes parathyroid gland aplasia [121, 128, 129].

[127, 140, 143]. Several patterns of thyroid dysfunction can occur in preterm neonates [140, 144, 145]:

1) Transient hypothyroxinaemia of prematurity: Occurs in 50% of preterm infants <28 weeks' gestation, most likely due to immaturity of the hypothalamic–pituitary axis. A postnatal TSH surge is evident at all gestational ages but is attenuated in extreme preterm infants. The biochemical picture is one of low free T_4 and normal TSH, and treatment is not needed.
2) Primary hypothyroidism: Very low birth weight (VLBW) infants have a higher risk of primary hypothyroidism and can exhibit a pattern of delayed TSH rise. In a considerable number of these infants, the hypothyroidism is transient. Treatment with TH should be initiated and continued until at least 3 years of age when TH-dependent brain maturation is complete. Thyroid function should then be reassessed at this stage.
3) Hypothyroidism due to iodine excess: Preterm infants are at risk from iodine-containing antiseptics and contrast agents.
4) Hypothyroidism due to iodine deficiency: Preterm infants are at risk of iodine deficiency as they have low iodine stores (usually established in the third trimester) and enteral and parenteral nutrition contains little iodine.
5) Thyroid dysfunction due to non-thyroidal illness: The biochemical picture is of low T_4/FT_4, T_3/FT_3 and TSH. Treatment is not needed. Repeat testing every 1–2 weeks is recommended.

Thyroid Hormone Stimulation of Programmed Fetal Development

The actions of TH on fetal tissues early in embryological development are emerging, with mounting evidence for the importance of their roles. Regulation of gene transcription is mostly via T_3, which binds to nuclear receptors encoded by the *THRA* and *THRB* genes. Thyroid hormone receptors (TRs) are transcription factors regulated by their ligands. TRs act as a molecular switch, controlling the timing of maturation in most tissues that are responsive [146, 147]. In the absence of T_3, the unliganded receptor (aporeceptor) recruits corepressors, repressing gene transcription. Non-T_3-binding receptors can also repress transcription by inhibiting receptor DNA binding. The maturation of tissues locally is initiated by T_3 availability, liganded T_3 receptor, T_3-mediated receptor exchange of corepressor with coactivators for creation of an active holoreceptor, and activation of responsive gene transcription. The iodothyronine deiodinases initiate or terminate TH action and are therefore critical for the biological effects mediated by TH. The activating deiodinase (D2) and the inactivating deiodinase (D3) can locally increase or decrease TH signalling in a tissue- and temporal-specific fashion independently of changes in TH serum concentrations.

These programming events have been investigated in studies of transgenic mice, including brain, liver, heart, intestine and bone tissues, thermogenesis and spleen erythropoiesis [146, 148–153]. Their timing in the mouse ranges from early midbrain neuronal development at gestational day 15, through perinatal activation of hepatic enzymes, cardiac ion channels and spleen erythropoiesis, to postnatal brain development, intestinal function and bone maturation and thermogenesis.

In hypothyroid mice the effects of repressive aporeceptors result in delayed maturation of tissues including the brain, heart, bone, spleen and intestine [152]. Development of vision and hearing stimulated by TH appears to be triggered by local expression of D2. This mediates local T_3 production, postnatally in the mouse and most likely towards the end of the second trimester in the human fetus [154]. At the time of parturition in mice and humans, an increase in circulating T_3 is seen, triggering maturation of tissue functions that are essential to enable transition to postnatal life (e.g. hepatic, cardiac and intestinal functions and brown fat thermogenesis).

In humans, T_3-mediated maturation of fetal tissues, including liver, heart, brown adipose tissue (BAT) and bone, allows them to become TH responsive during late gestation and in the perinatal period [155]. Paracrine actions of TH are critical for normal fetal development, for example, in the cochlea, where D2 is expressed in connective tissue immediately adjacent to the sensory epithelium and spiral ganglion, where TRs are located. This implies that D2-containing cells in the connective tissue take up T_4 from the circulation, convert it to T_3 and then release D3 to adjacent responsive cells. Similarly, in the brain D2 is expressed predominantly in glial cells [156], whereas TRs are expressed in adjacent neurons and oligodendrocytes. In other areas of the brain, such as the pituitary gland, hippocampus and caudate nucleus, there is co-expression of D2 and TRs. On the other hand, D3 is co-expressed with TRs in neurons, thereby protecting sensitive tissues from the effects of excess TH.

The actions of THs and their developmental regulation in the brain are complex. Functionally, THs are essential for the establishment of neural circuits during a critical window of brain development [155, 156]. They provide inductive cues for many processes including neurogenesis and neural cell migration (occurring between 5 and 24 weeks' gestation), neuronal differentiation, dendritic and axonal growth, synaptogenesis, gliogenesis (occurring from late third trimester to 6 months' post-partum), myelination (occurring from second trimester to 24 months' post-partum) and

neurotransmitter enzyme synthesis. TRs are found in highest concentration in developing neurons and in multiple areas of the fetal brain, including the cerebrum, the cerebellum and the auditory and visual cortex [156]. The hormones bind to receptors and stimulate genes such as myelin, neurotrophins and their receptors, cytoskeletal components, transcription factors, extracellular matrix proteins and adhesion molecules, intracellular signalling molecules and mitochondrial and cerebellar genes.

Normal bone growth is also reliant on TH. TRs are expressed on osteoblasts and growth plate chondrocytes and T_3 target genes have been identified in bone. Endochondral ossification and chondrocyte differentiation in the growth plate are regulated by T_3 both *in vivo* and *in vitro*. T_3 stimulates closure of skull sutures *in vivo* [157].

TH in the perinatal period is important for homeostasis. Transcription of thermogenin, also known as uncoupling protein 1 (UCP1), is stimulated by TH. UCP1 uncouples nucleotide phosphorylation and the storage of energy as adenosine triphosphate (ATP), which is important for non-shivering thermogenesis by BAT [158].

Congenital Hypothyroidism

Congenital hypothyroidism does not characteristically present in the immediate neonatal period, although it is rarely associated with respiratory distress in the newborn. The classical signs of congenital hypothyroidism include lethargy, constipation, jaundice, feeding difficulties, macroglossia, myxoedema, hypothermia, poor growth and progressive developmental delay. These appear during the initial critical weeks and months of postnatal life as maternal T_4 becomes unavailable and non-CNS tissues become responsive to TH [22, 122]. TH is critical for neurodevelopment, particularly in the first 3 years of life and most countries have a robust screening programme to ensure early diagnosis and treatment [159]. The mechanisms resulting in congenital hypothyroidism due to abnormal thyroid development, dyshormonogenesis, abnormal TH transport or action and genes involved in these mechanisms, as well as management, are covered in detail in Chapter 8.

Gonadal Development

The undifferentiated bipotential gonad is directed to become a testis or ovary following a series of molecular events resulting in sex determination. Subsequently sex differentiation takes place through factors produced by the gonads, and these factors govern the development of phenotypic sex. Seminal in the field of fetal sex development was the work of Alfred Jost, who studied the mechanism of somatic sex differentiation by examining the pathophysiology of an experiment of nature, the freemartin calf.

The freemartin phenotype appears in a dizygotic twin pregnancy, where a genetically female fetus is masculinized in the presence of a male twin. The phenomenon of 'freemartinism' is a disorder of sex development (DSD). It is explained by the conjoined circulations of twin calves due to placental vascular anastomoses seen in cattle. This enables testosterone and anti-Müllerian hormone (AMH) from the male calf to affect the genital development of the female calf. Subsequent advances in understanding the genetic regulation and pathophysiology of sex development have revealed that there is potential for disruption of the sex differentiation process at different stages, and clinical phenotype will depend on the nature of the disruption.

Gonadal Embryology

The mammalian gonad originates from the intermediate mesoderm, from which the urogenital ridge differentiates. The urogenital ridges are the common precursors of the urinary and genital systems and adrenal cortex. Each develops at the ventral surface of the cranial mesonephros and divides into a urinary and an adrenogonadal ridge, which later gives rise to the gonad and adrenal.

The gonadal ridge is bipotential and can develop into an ovary or a testis. Several genes, including those encoding *NR5A1*, *WT1*, *EMX2* (empty spiracles homeobox 2), *CBX2* (chromobox 2) and *PBX1*, are required for formation of the bipotential gonadal ridge [160]. The gonad is derived from two tissue anlagen, the primordial germ cells of the yolk sac wall and the somatic stromal cells that migrate from the primitive mesonephros [161, 162]. Germ cells migrate from the yolk sac by 2–3 wpc and are incorporated into the developing gonadal ridge from 4 wpc. The primitive gonad comprises a surface epithelium, primitive gonadal cords continuous with the epithelium and the AGP [162]. As the AGP grows, the cells delaminate from the coelomic epithelium and invade the underlying mesenchyme, with the cells adjacent to the mesonephros migrating dorsolaterally to form the gonadal primordium.

The development of a specific sex phenotype requires the action of networks of transcription factors and complex signalling cascades that regulate cell fate and differentiation of the bipotential gonad into a testis or ovary from 6 wpc. The fetal gonads are indistinguishable until this point, when testicular cords composed of pre-Sertoli cells then appear.

The identification of a testis-determining gene, *SRY* (sex-reversed on the Y), was an important step towards elucidating the genetic pathway for gonadal development. It is now clear that numerous other genes are necessary for normal testis development, including *NR5A1*, *SOX9* and *NR0B1* [163, 164]. *SRY*, thought to have evolved from *SOX3*, is a critical regulator of male gonadal

differentiation. The X chromosomal *SOX3* gene is not normally expressed in the developing gonad. Therefore, when ectopically expressed, it might substitute for *SRY* in driving testicular development. Loss-of-function mutations of *Sox3* do not affect sex determination but *Sox3* overexpression in murine XX gonads leads to testis differentiation and, in humans, *SOX3* duplication or rearrangements of the *SOX3* regulatory region have been found in 46,XX individuals with dysgenetic testes [165].

NR5A1 mediates *AMH* gene expression and gonadotropin production and is necessary for testicular and ovarian development. *SRY* is expressed in pre-Sertoli cells in the XY gonad, by 7 wpc. This results in up-regulation of *SOX9* expression which is further augmented by the synergistic action of *SRY* and *NR5A1*, leading to definitive differentiation of Sertoli cells. The binding of *SRY* and *NR5A1* to the testis-specific enhancer of SOX9 core (TESCO) region, which lies approximately 13 kb upstream of *SOX9*, mediates transcriptional activation. Genomic rearrangements (such as duplication and triplication) affecting *SOX9* regulatory elements, located significantly further upstream (500 kb) than the TESCO enhancer region, have been found in patients with 46,XX sex reversal [160].

Once a critical threshold of *SOX9* expression is reached, autoregulation of *SOX9* transcription occurs, and feedforward loops are formed via downstream signalling pathways such as fibroblast growth factor 9 (FGF9) and prostaglandin D2 (PGD2). *SOX9* regulates AMH production from testicular Sertoli cells and may repress genes involved in ovarian development such as *WNT4* (wingless-type MMTV integration site family, member 4) and *FOXL2* (forkhead box L2). *DMRT1* (Doublesex and mab-3 related transcription factor 1) may also play a role in this process [160].

WT1, located at 11p13, is also critical for normal male sex differentiation, and is expressed in the bipotential gonadal ridge. *WT1* isoforms act synergistically with *NR5A1* to increase expression of AMH. In addition, WT1 binds to and activates the *SRY* promoter. *WT1* missense mutations associated with 46,XY DSD in Denys–Drash syndrome, result in failure to synergize with *NR5A1* [166].

SRY expression is transcriptionally regulated by *WT1*, *NR5A1*, *GATA-4* and its cofactor zinc finger protein *FOG-2* (ZFPM2), and chromobox protein homologue 2 (*CBX2*) [167]. In the presence of *SRY*, male gonadal differentiation is initiated with organization of the gonadal blastema into interstitium and germ cell-containing testicular cords. The primitive Sertoli cells and spermatogonia become visible within the cords, and the epithelium differentiates to form the tunica albuginea [168]. Via a hedgehog signalling pathway, which produces androgens and INSL3 (a member of the insulin-like family), Sertoli cells induce the development of Leydig cells at 8–9 wpc [169]. INSL3 is necessary for testicular descent [160] and rare mutations have been described in cryptorchidism [170, 171]. Regression of Müllerian structures and differentiation of the Wolffian duct into the epididymis, vas deferens and seminal vesicles occur under the influence of testosterone and AMH. In 46,XY males, testosterone is converted to 5-dihydrotestosterone (DHT) by the enzyme 5-alpha reductase. DHT is a potent agonist of the androgen receptor (AR), and binding of this ligand results in development of male external genitalia.

At 12 wpc growth of the fetal testes occurs, with an increase from 20 to 800 mg at birth. Testicular descent into the inguinal canal in association with the vas deferens and epididymis occurs at around 5–6 months [168]. The fetal gonad, adrenal and kidney all initially develop in close proximity and as the testes descend, they may carry rests of adrenocortical cells with them. These adrenal rests can hypertrophy, resulting in testicular enlargement, if subjected to prolonged ACTH stimulation (for example, in patients with poorly controlled CAH).

Ovarian differentiation begins at 5 wpc, in the absence of *SRY*. In the XX gonad, the absence of *SRY* results in failure of *SOX9* expression to reach a critical threshold. Together with the expression of factors such as R-spondin 1 *(RSPO1)/WNT4* signalling, *FST* (follistatin) and *FOXL2* (forkhead box L2) lead to formation of the ovary. In part this occurs via suppression of the activity of 'testis' genes. In the 46,XX female, the absence of androgens leads to the development of female genitalia; Wolffian duct regression is seen and the Müllerian duct is maintained forming the oviduct, uterus, cervix and upper part of the vagina [160]. The gonadal blastema differentiates into interstitium and medullary cords which contain the primitive germ cells (oogonia). The cords degenerate and cortical layers of surface epithelium, containing individual small oogonia, appear. By 9–10 wpc, clusters of dividing oogonia are surrounded by cord cells within the cortex and the medulla consists largely of connective tissue [172]. From 10 wpc, primitive granulosa cells begin to replicate and many of the large oogonia in the deepest layers of the cortex enter their first meiotic division. Oogonia develop in clusters of cells called germline cysts or oocyte nests and then enter meiosis and become oocytes. The oocyte nests break apart into individual cells and become packaged into primordial follicles.

Primordial follicles are evident at about 16 wpc and subsequently numbers increase rapidly [173]. However, the number of oocytes decline, from a peak of 3–6 million at 5 months' gestation to approximately 2 million at term [59, 173]. Germ cell proliferation and apoptosis occur simultaneously, and although oocytes cluster, these break down with the development of follicles. Only those oocytes enfolded by developing granulosa cells as

primordial follicles manage to survive [59, 173]. During the seventh month of gestation, stroma-derived theca cells surround the primordial follicles as they mature forming primary follicles, and this process continues after birth.

Each fetal ovary weighs approximately 15 mg at 12 wpc and this increases to 300–350 mg by term [172]. The number of surviving primary follicles at birth correlates with the duration of subsequent post-pubertal ovulation. Interstitial cells with characteristics of steroid-producing cells are present after 12 wpc, and during the third trimester, theca cells with steroidogenic capacity surround the developing follicles [59]. Significant aromatase activity is also present but few, if any, steroids are produced by the ovary during fetal development [59, 172].

The underlying genetic mechanisms dictating ovarian development are being elucidated, and some of the most influential regulators include the WNT/FZD (Frizzled)/β-catenin, FOXO/FOXL2 (forkhead box protein O/forkhead box protein L2) and TGF-β/SMAD (transforming growth factor β/small mothers against decapentaplegic) pathways [167, 174]. *FOXL2*, encoding a forkhead transcription factor, is required for ovarian development [175] and *DMRT1*, expressed in testis, prevents expression of *FOXL2*, and hence female programming, in the postnatal testis [176]. In the XX gonad, *RSPO1*, *WNT4*, *CTNNB1* (catenin beta 1), *FOXL2* and *FST* are also expressed in a female-specific manner to promote ovarian development and repress testicular development. In humans and mice, *RSPO1* augments β-catenin signalling, possibly via *WNT4* [177]. A syndrome with palmoplantar hyperkeratosis and 46,XX DSD with sex reversal and dysgenetic testes or ovotestes has been described due to homozygous mutations in *RSPO1* [178]. Mutations in *WNT4* are associated with Mayer–Rokitansky syndrome and SERKAL (sex reversal, dysgenetic kidneys, adrenals and lung) syndrome [173, 179].

Fetal Gonadal Sex Steroid Production

In the male fetus, the development of Leydig cells leads to an increase in fetal testosterone production between 8 and 18 wpc (Figure 3.9) [53, 168, 180–182]. AR appear in the epithelium during development at 7–10 wpc [179], with no difference in expression evident between sexes.

In vitro, hCG binding to rat fetal testis cells does not down-regulate LH receptors. Fetal LH may contribute to fetal Leydig cell function, but quantitatively hCG is the predominant gonadotropin. Testosterone itself, acting through the AR, stimulates differentiation of the primitive mesonephric ducts into bilateral vas deferens, epididymides, seminal vesicles and ejaculatory ducts. DHT stimulates male differentiation of the urogenital sinus and external genitalia, including differentiation of the prostate, growth of the genital tubercle to form a phallus, and fusion of the urogenital folds to form the penile urethra. DHT mediates the action of testosterone in the Wolffian ducts.

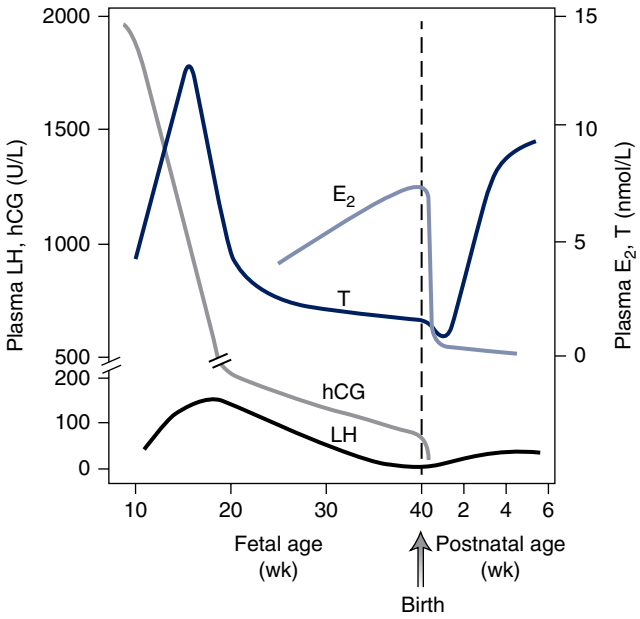

Figure 3.9 Patterns of change of plasma concentrations of human chorionic gonadotropin (hCG), luteinizing hormone (LH), testosterone (T) and oestradiol (E_2) in a male fetus during gestation and in the neonatal period. *Source:* Data from Penny et al. [180], Reyes et al. [181], Kaplan et al. [53], and Forest and Cathiard [182].

The fetal testis also produces AMH, which causes dedifferentiation of the Müllerian duct system in the male fetus [183, 184]. AMH is produced by testicular Sertoli cells and reaches the Müllerian ducts largely by diffusion; duct regression *in vitro* requires 24–36 hour exposure to AMH. AMH is produced early in gestation, peaking at the time of Müllerian duct regression; thereafter, synthesis continues throughout gestation and decreases after birth. *AMH* gene expression is activated by the *SRY* and *SF-1* genes [183]. Additionally, AMH has autocrine and paracrine effects on testicular steroidogenic function during fetal life [184]. Male phenotypic differentiation is mediated by testicular testosterone and AMH and occurs between 6 and 12 wpc.

In the absence of AMH, in the female fetus the Müllerian duct system differentiates, the mesonephric ducts fail to develop in the absence of testosterone, and the undifferentiated urogenital sinus and external genitalia mature into female structures. Mutation of the *AMH* gene results in a persistent Müllerian duct syndrome in the XY fetus [183].

Oestrogen effects are mediated by cognate receptors [185, 186]. Two oestrogen receptors (ERs), ER*α* (encoded by *ESR1* on chromosome 6) and ER*β* (encoded by *ESR2*

on chromosome 14), have been identified, with 96% and 58% homology in the DNA-binding and ligand-binding domains, respectively. Expression of these receptors has been characterized in the 14–21 wpc fetus and one or both receptor mRNAs are present in most tissues. The *ESR2* transcript is predominant, particularly in testis, ovary, spleen, thymus, adrenal, brain, kidney and skin. The *ESR1* transcript is prominent in the uterus, with relatively low levels in most other tissues [185, 186].

Knockout of the *Esr1* gene in mice does not impair fetal development of any tissue but adult females are infertile with hypoplastic uteri and polycystic ovaries and adult males manifest decreased fertility [186]. *Esr2* knockout mice develop normally and female adults are fertile with normal sexual behaviour; adult males reproduce normally but have prostate and bladder hyperplasia [185].). The significance of ERs in fetal development is unclear, however it is known that oestrogens regulate DHEA production in the baboon and HFA [185].

Knockout of both *Esr1* and *Esr2* genes has little impact on fetal development but, after birth, the uterus, fallopian tubes, vagina and cervix in females are hypoplastic and unresponsive to oestrogen [186]. In humans, *ESR1* mutations in males are associated with tall stature, insulin insensitivity, and osteoporosis [187].

Androgens and oestrogens are both involved in the structural development of the rat brain [188]. Gonadal hormones also control gonadotropin production in the brain that results in cyclic ovarian function and normal function of the testes [189, 190]. Testosterone administration to neonatal female rats produces permanent inhibition of cyclic hypothalamic control through local aromatization to oestradiol and ER binding. In primates and humans, oestrogens seem to be more effective in this regard, although the mechanisms for these effects are not yet clear in the fetus. Furthermore, there is no evidence for permanent programming in the primate and there appear to be no major tissue biochemical differences between the sexes *in utero* to account for sexual dimorphic behavioural or gonadotropic programming [189].

A current view of the pathways for genes programming gonadal differentiation is shown in Figure 3.10 [163, 164]. The full spectrum of downstream gene targets remains to be defined but the net result is the highly organized pattern of gonadal development and phenotypic sexual differentiation. Fetal pituitary gonadotropins are not required for gonadal development or sexual differentiation; LH or FSH receptor knockout mice are born phenotypically normal [191].

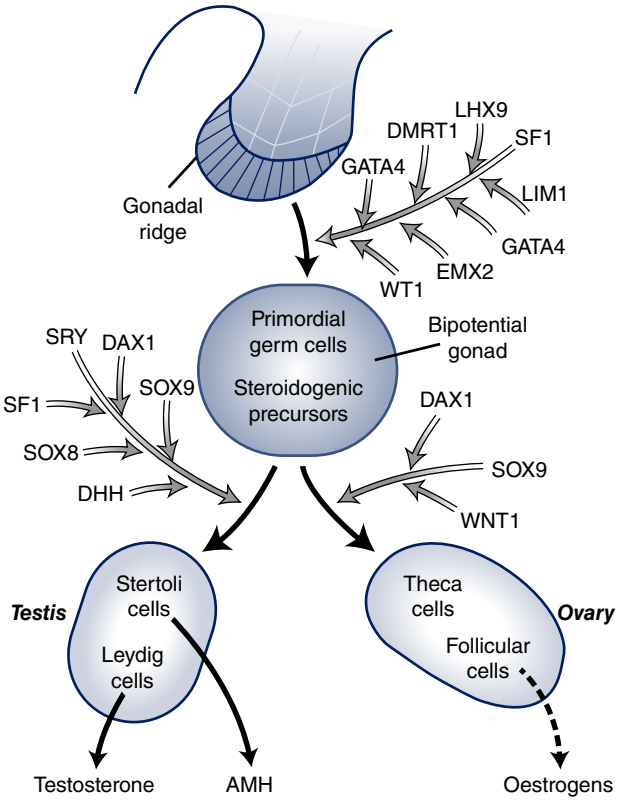

Figure 3.10 Summary of the molecular and cellular events of gonadal differentiation. AMH, anti-Müllerian hormone or Müllerian-inhibiting substance; DHH, desert hedgehog. Molecular cascades developed from Harley et al. [163] and Park and Jameson [164].

Disorders of Sex Development (see Chapter 4)

Optimal care for infants with DSD requires an experienced multidisciplinary team that transitions into adulthood, and there is a need for greater data sharing and formal research and clinical expertise networks, as highlighted by the Chicago Consensus in 2005. The growing knowledge about sex determination and differentiation and society's evolving fluidity about the nature of sexual identity and gender roles have led to reappraisal of former management practice in DSD. Human mutations in several genes programming gonadal differentiation are described in detail in Chapter 4 and it is likely that future innovations in technology will lead to improved understanding of underlying abnormalities as well as explain the variability in the phenotype.

Development of the Fetal Autonomic Nervous System

The development of the autonomic ability to regulate the fetal CNS and adapt to perturbations in homeostasis is a key necessity during human fetal maturation. The interplay between sympathetic and parasympathetic nervous systems is achieved via the cerebral cortex, the medulla oblongata, the sympathetic ganglia and the vagus nerve. This process of fetal autonomic regulation *in utero* serves

highly specialized needs throughout gestational development and reaches a pace that may never be observed again postnatally. Nowhere is this more evident during fetal development than in the sympathetic nervous system and its endocrine counterpart, the adrenal medulla.

Embryology of the Autonomic Nervous System

Primordia of the sympathetic trunk ganglia are visible from as early as 4 wpc in the human fetus. Sympathoadrenal (SA) cells constitute a major lineage among neuroectodermal crest (NC) derivatives, giving rise to sympathetic neurons, neuroendocrine chromaffin cells of the adrenal medulla, and extra-adrenal chromaffin cells [192]. This implies that NC cells are instructed to an SA cell fate and that local signals are important for differentiation of SA cells into sympathetic neurons and chromaffin cells. The mechanisms are largely unknown. SA progenitor cells that migrate dorsolaterally form extramedullary sympathetic paraganglia [192], which are located throughout the abdominal and pelvic sympathetic plexuses [193]. Each paraganglia may reach 2–3mm diameter by 28 wpc, and the largest of these, the organs of Zuckerkandl located near the origin of the inferior mesenteric arteries, may reach 10–15 mm at term. After birth, the paraganglia slowly atrophy and disappear by 3 years of age.

SA cells that migrate ventrally to reach the dorsal aorta give rise to tyrosine hydroxylase-expressing catecholaminergic neuronal cells [194]. This occurs in response to bone morphogenetic protein (BMP) cues from cells of the wall of the dorsal aorta and the surrounding mesenchyme [195]. There is migration of some cells from the dorsal aorta to enter the cranial end of the AP from 6 wpc [196, 197], acquiring a chromaffin cell phenotype. At least some of these SA cells enter the adrenal still expressing NC markers and then gain tyrosine hydroxylase and dopamine β-hydroxylase expression, but not neuronal markers [198]. Numerous transcription factors have been identified in the development of the SA cell lineage including *PHOX2B* (paired-like homeobox 2b), *MASH1* (mammalian achaete scute homologue-1), *PHOX2A* (paired-like homeobox 2a) and *HAND2* (heart- and neural crest derivatives-expressed protein 2) [192]. *PHOX2B* has been demonstrated to be pivotal in the development of the autonomic nervous system. *Phox2b*-null mice die *in utero* since autonomic nervous system neurons either fail to develop or degenerate. Mutations in human *PHOX2B* cause a rare syndrome of dysautonomia (congenital central hypoventilation syndrome) and a predisposition to neuroblastoma [199].

Nerve growth factor (NGF) is required for the survival of developing sympathetic neurons. In the rodent. NGF antiserum injected into neonatal rats results in degeneration of immature chromaffin cells, and sympathetic cells [200]. It is unclear whether NGF and other growth factors play a role in the maturation of the human fetal autonomic nervous system.

Sympathoadrenal Development

The endocrine cells of the adrenal medulla are referred to as chromaffin cells due to characteristic darkening of the tissue on exposure to chromium salts.in aqueous solution. Chromaffin cells are located in adrenal and extra-adrenal sites during fetal development, although the function of the extra-adrenal tissue is unknown and these chromaffin cells regress postnatally. Chromaffin cells are initially scattered within the adrenal and are mostly noradrenergic. At around 10 wpc, small islets of chromaffin cells express phenylethanolamine *N*-methyltransferase (PNMT), the enzyme required to convert noradrenaline (NA) into adrenaline, to become adrenergic [201]. With increasing gestational age, there is progressive growth of the adrenal medullae, increasing catecholamine content and progressive maturation of function. Histologically, the adrenal medullae are somewhat immature at birth but by 1 year they resemble the adult adrenal.

Catecholamines are present in the para-aortic chromaffin tissue by 8–13 wpc and concentrations increase until term. The predominant catecholamine is NA, presumably because of low activity of PNMT in para-aortic chromaffin tissue. In contrast, the adrenal medulla has high levels of PNMT activity. Adrenal PNMT is activated by glucocorticoids that diffuse into the adrenal medulla from the adrenal cortex [193, 202], and cortisol and catecholamine concentrations in the fetus increase significantly in the third trimester, playing a critical role during transition of the fetus to an *ex utero* environment by priming the fetal nervous system. How the fetus withstands an *in utero* environment of relative hypoxia has been a large area of research for over a century. The ovine model provides a comparative system for physiological and developmental research in the fetus that is applicable to human. Basal plasma adrenaline, NA and dopamine concentrations decrease during the last trimester in sheep [203, 204] due to maturation of clearance mechanisms [204]. This model has been used to study fetal adaptive responses to chronic oxygen and nutrient deprivation during placental insufficiency. The fetal sheep responds to hypoxia with increased catecholamine concentrations [205]. This response is also present in human fetuses during the third trimester [206]. The fetal adrenal and the para-aortic chromaffin tissues discharge large amounts of catecholamines directly into the circulation in response to fetal hypoxia [202]. Moreover, the defence against fetal hypoxia involves catecholamine actions mediated through cardiac α-receptors that are unique to immature animals. α-Adrenergic receptors predominate in immature cardiac tissue and gradually decline in number as β-adrenergic receptors

increase with maturation. Chromaffin tissue in the fetus is also innervated by opiate receptors and contains relatively large amounts of opiate peptides that appear to be co-secreted with the catecholamines [202]. Central and adrenal enkephalins are involved in fetal autonomic nervous system function; pretreatment with naloxone potentiates and methadone inhibits the catecholamine response to hypoxia [193, 207]. The extent to which these peptides are involved in modulating fetal catecholamine secretion remains unclear.

Catecholamines are critical for fetal cardiovascular function and survival. Gene knockout studies in mice, targeting either tyrosine hydroxylase or dopamine β-hydroxylase, produce fetal catecholamine deficiency and mid-gestation fetal death was seen in 90% of the mutant embryos [208, 209]. Additionally, fetal catecholamines are the major stress hormones in the fetus [202, 206, 207, 210]. The human neonate responds to parturition with an increase in plasma adrenaline and NA concentrations and these responses are augmented by hypoxia and acidosis [205, 208, 209]. In the newborn infant, catecholamine secretion also increases after cold exposure and hypoglycaemia [202, 206]. Labour-induced catecholamine release may provide an important mechanism underlying neonatal adaptation to extrauterine conditions.

Development of the Endocrine Pancreas

Pancreatic Embryology

Pancreatic development is a intriguingly complex multistep process, in which morphologically and functionally distinct exocrine and endocrine tissue types are derived from common endodermal progenitor cells. Exocrine tissue comprises acinar cells that secrete digestive fluid, centroacinar cells and a duct system by which the fluid drains into the intestine. The endocrine tissue is arranged as discrete islets of Langerhans, which comprise multiple distinct cell types secreting at least five different hormones into the circulation (α-cells, glucagon; β-cells, insulin; δ-cells, somatostatin; ε-cells, ghrelin; and γ [or PP]-cells, pancreatic polypeptide).

Our understanding of human pancreatic development has on the whole been extrapolated from data obtained in other species, particularly mouse. Pancreatic embryogenesis is mediated by a series of homeobox genes and transcription factors. These program pancreatic budding from the gut tube, development of branching ducts and undifferentiated epithelium, differentiation of exocrine and endocrine cell lineages, and organization of the endocrine cells into islets of Langerhans [211]. After gastrulation, the earliest patterning event leading to pancreatic development is the exclusion of *SHH* from the dorsal endoderm where it contacts the notochord. This was originally described in the chick and is mediated in part by activin signalling from the notochord. Members of the EGF family of growth factors, laminin, and perhaps other growth factors including the IGFs also contribute to pancreatic growth and differentiation [212, 213].

Cell lineage determination in the mouse commences on day 8 of the 21-day gestation and continues until 2–3 weeks after birth (Figure 3.11) [214]. Several transcription factors have been shown to be critical for the establishment and proliferation of multipotent pancreatic progenitors. The anterior endoderm invaginates to form the anterior intestinal portal (AIP), which marks the foregut–midgut boundary, and is the site of pancreas specification (which occurs at E7.5 in mouse). Pancreas induction occurs at the equivalent place and is very similar, but slightly delayed, in human embryos, with endodermal folding (and thus formation of the AIP) apparent at Carnegie Stage (CS) 10 (corresponding to approximately E8–E8.5 in mouse) [215].

Exclusion of *Shh* allows expression of the key transcription factor pancreatic and duodenal homeobox factor 1 (*pdx1*) in these cells, without which the pancreas is unable to form [215]. In human embryos, *SHH* can be detected at CS10 and *PDX1* at CS12, and the phenotype in mice is mimicked in humans with homozygous loss of *PDX1* resulting in pancreatic agenesis. *Pdx1* expression is followed by expression of the basic helix-loop-helix protein pancreas specific transcription factor 1a (*Ptf1a*), necessary for further development of both the endocrine and exocrine pancreas, forkhead box O1 (*Foxo1*), Nirenberg and Kim homeobox factor 2 homeobox 2 (Nkx2.2) and NK6 homeobox 1 and 2 (Nkx6.1 and Nkx6.2, respectively) [215, 216]. Regulatory factor X 6 (*Rfx6*) is also expressed during these early time points in mice, but it appears only to be required for endocrine pancreatic development, a phenotype shared in humans with mutations in *RFX6* [215].

In human pancreatic development, the ventral and dorsal pancreatic buds at CS13 are marked by the transcription factors *SOX9*, *PDX1* and GATA binding protein 4 (*GATA4*). The dorsal bud at CS13 is also characterized by the appearance of microlumens by which acinar secretions will eventually drain into the intestine. For the remainder of the embryonic period, the human pancreas undergoes a large expansion of proliferative progenitor cells. In human, unlike in mouse, NKX2.2 protein is not detected in these cells.

Motor neuron and pancreas homeobox 1 (*Mnx1*), formerly known as *Hlbx9* or *Hb9*, is expressed early but, unlike the previously mentioned transcription factors, *Mnx1*-null mice fail to develop a dorsal pancreas and the resulting pancreas has fewer and dysfunctional islets [211, 214, 215]. This phenotype is observed in humans with mutations in *MNX1*.

Loss of hes family BHLH transcription factor 1 (*Hes1*), a notch signalling pathway component, in mice disrupts normal development of the pancreas; however, formation of exocrine and endocrine cells still occurs [216]. During this period, the only endocrine cells produced are a small number of glucagon-positive cells, and it is unclear whether these play a role in development and postnatal function [211].

Expression of the basic helix-loop-helix transcription factor neurogenin 3 (*Ngn3*), at around E13.5 in the mouse, marks the endocrine progenitor cells that will differentiate into the mature hormone-producing cells of the islets of Langerhans [211, 215, 216]. *Ngn3* is only expressed before hormone expression, and loss of *Ngn3* prevents the development of endocrine progenitor cells and consequently pancreatic islets. *Ngn3* knockout in the mouse leads to marked β-cell dysplasia and hypoplasia [214]. During human pancreatic development, *NEUROG3* expression increases immediately after the embryonic period timed with the appearance of fetal β-cells, the first predominant islet cell type to appear. *SOX9* is absent in cells with robust *NEUROG3* concentrations and is not detected in endocrine cells thereafter, although it does persist in pancreatic duct cells

Ngn3 initiates a new set of developmental programmes for endocrine cell lineage differentiation and maintenance. These include the LIM homeobox protein islet-1 (*Isl1*), paired box 4 and 6 (*Pax4* and *Pax6*), aristaless related homeobox (*Arx*) and neuronal differentiation 1 (*Neurod*), which are all lost in *Ngn3*-deficient mice. In mice, *Ngn3* knockout leads to complete absence of endocrine cells, whereas knockout of the lower pathway genes shown in Figure 3.11 impairs specific islet cell differentiation [214]. *Nkx2.2*, *Nkx6.1*, *Pax-4*, or *Pax-6* loss of function results in endocrine cell agenesis or hypogenesis [217–219]. The competency of progenitors changes with time such that Ngn3+ cells that arise early in development preferentially differentiate into glucagon+ cells while later Ngn3+ cells give rise to insulin+ or SS+ cells [215].

The *MafB* and *MafA* (V-maf musculoaponeurotic fibrosarcoma oncogene homologue B and A) transcription factors play a prominent role during the later stages of β-cell differentiation. Upon β-cell specification, the transcription of insulin is initiated and maintained by *MafB*, *Pdx1*, *Neurod1*, *Pax6* and *MafA*. Additionally, *Pax4* and *NKX6*.1 and *Pdx1* inhibit glucagon expression, preventing the expression of glucagon by β-cells [215].

Arx is one of the earliest transcription factors specifying the glucagon-producing α-cell lineage and its loss prevents development of α-cells with a simultaneous increase in β- and δ-cells [215]. A reciprocal interaction between *Arx* and *Pax4* is involved in the decision of cell fate between α- and β-cells. *Arx* is necessary for the early

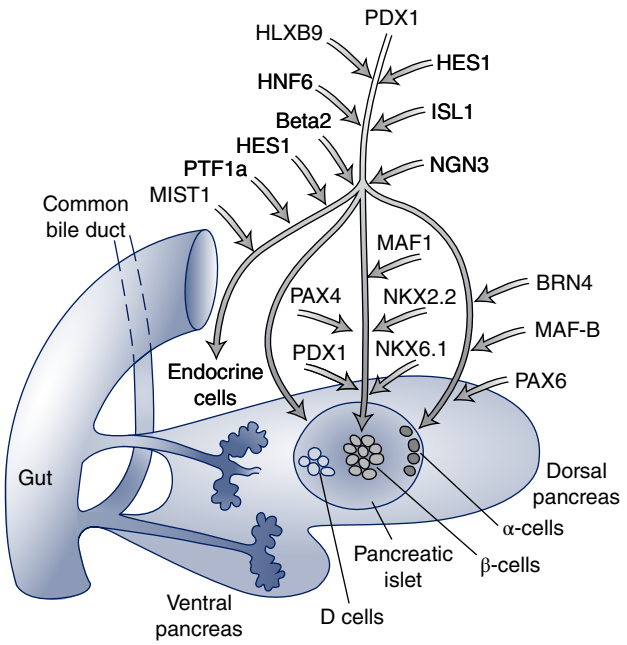

Figure 3.11 Expression of main transcription factors during pancreatic embryogenesis. Knockout of *PDX1*, *MNX1* (previously called *HLXB9*) or *ISL1* is associated with early arrest of pancreatic development. *MNX1* knockout leads to failure of the pancreatic dorsal bud to develop with decreased β-cell number in the remnant pancreas. *HES1*, *PTF1A* or neurogenin 3 (*NGN3*) disruption leads to aplasia or hypoplasia of the islets of Langerhans. Disruption of the downstream transcription factors impairs formation of the β-cells or α-cells. *SOX9* and *HNF3B* (not shown) are required for early foregut formation and pancreas specification. *Source:* From Habener et al. [214]. Reproduced with permission of Oxford University Press.

specification of the α-cell and directly maintains α-cell mass. The Pax4-related factor *Pax6* is expressed by both α- and β-cells, and *Pax6*-deficient mice lack α-cells. In addition to its role in insulin transcription in the β-cell, *Pax6* also coordinates glucagon transcription in the α-cell directly by binding to the glucagon promoter and indirectly by inducing expression of other transcription factors such as *c-Maf*, *MafB* and *Neurod1*, which also activate glucagon expression.

Forkhead box A2 (*Foxa2*), which plays a role downstream of *Arx* and *Pax6*, is the third factor that is crucial for later α-cell development [215]. A *de novo* heterozygous mutation in *FOXA2* has been described in a child with congenital hyperinsulinism (HI) and congenital hypopituitarism with craniofacial dysmorphic features, choroidal coloboma and endoderm-derived organ malformations in liver, lung and gastrointestinal tract [220]. Expression profiling in human embryos by immunohistochemistry showed strong expression of *FOXA2* in endoderm-derived organs including the pancreas, and transfection studies and Western blot assays demonstrated the causative role of FOXA2 in this syndrome.

Upon successful specification of the α-cell lineage, pre-pro-glucagon expression is further promoted by forkhead box A1 (*Foxa1*), Brain 4 (*Brn4*) and *Isl-1*. Of all the transcription factors that can promote glucagon expression, expression of only two, *Brn4* and *MafB*, is enriched in adult mouse α-cells compared to β-cells [215].

More recent studies have compiled gene regulatory networks for pancreas development. These networks include the previously mentioned genes but also others such as *Tle2*, *Dll*, *Onecut1* and *BMP7* [218]. Expression of *Ngn3*, *Arx* and Gli-similar 3 (*Glis3*) expression is restricted to the later establishment of defined endocrine progenitor cells (or endocrine and ductal cells in the case of *Glis3*). The transcription factor *GLIS3* has been implicated in the development of neonatal diabetes [221]. Loss of functional *Glis3* leads to a severe reduction in both β- and PP-cell number postnatally, as well as formation of cystic pancreatic ducts. In humans, mutations in *NeuroD1*, *PDX1*, *PTF1A* and *GATA6* are associated with neonatal diabetes mellitus due to pancreatic agenesis. Mutations in *EIF2AK3* (encoding translation initiation factor 2-alpha kinase 3), *HNF1B* (hepatocyte nuclear factor-1-β), *MNX1*, *NKX2.2* and *RFX6* result in pancreatic hypoplasia causing neonatal diabetes [222–225]. Recessive mutations in a distal enhancer of *PTF1A* also cause pancreatic agenesis [226].

Development of Pancreatic Endocrine Function

The human fetal pancreas is evident as early as 4 weeks' gestation and α- and β-cells can be recognized by 6–7 wpc. Insulin, glucagon, somatostatin and PP are measurable by 6–8 wpc [227].

α-Cells and β-cells have opposing roles in the regulation of glucose homeostasis although they are derived from a common progenitor. Mouse models have shown that β-to-α as well as α-to-β transdifferentiation can take place under certain experimental circumstances, revealing plasticity among pancreatic endocrine cells [215]. In the first half of gestation, α-cells outnumber β-cells reaching a relative peak at mid-gestation. β-Cells continue to increase throughout the second half of gestation, so, by term, the ratio of α- to β-cells is approximately equal.

The β-cell is capable of producing insulin by 12 wpc, and throughout most of pregnancy, concentrations exceed adult β-cell production, which is only approximately 14 pmol/g (2 U/g). The insulin content of the pancreas increases from <3.6 pmol/g (0.5 U/g) at 5–8 wpc to 30 pmol/g (4 U/g) at 14–23 wpc and 93 pmol/g (13 U/g) near term [228]. Endocrine cells are scattered throughout the exocrine tissues by 18 wpc and the islets of Langerhans are clearly differentiated by 29 wpc.

Fetal glucose homeostasis is largely independent of insulin and glucagon [229–231], and acute hypoglycaemia or hyperglycaemia is not associated with significant changes in either insulin or glucagon concentrations. Although the fetal β-cell is functional by 12–22 wpc, secretion of insulin into the bloodstream is low. *In vitro*, insulin secretion from fetal rat pancreas in response to glucose is minimal; however it can be stimulated by leucine, arginine, tolbutamide or potassium chloride, indicating that elements of the secretory mechanism are functional in the fetus [228, 229, 232]. Insulin secretion in adult islets is mediated by voltage-gated calcium channel activation; however this does not occur in fetal islets [232]. In pregnant women, infusion of glucose or arginine fails to provoke fetal insulin secretion at mid-gestation or near term and plasma insulin concentrations in the human fetus are relatively unresponsive to the high glucose concentrations prior to onset of labour [228].

In the anencephalic human fetus, the endocrine pancreas develops normally provided maternal carbohydrate metabolism is not interrupted. β-Cell hypertrophy and hyperplasia do not occur in the anencephalic fetus exposed to chronic hyperglycaemia, perhaps due to deficiency of GH or IGF1 or both. GH stimulates insulin gene expression and may play a permissive role in β-cell hyperplasia and hypertrophy [213]. Chronic fetal hyperglycaemia does induce hyperinsulinaemia and glucagon suppression, and chronic hypoglycaemia may inhibit fetal insulin and promote fetal glucagon release [218].

Glucagon concentrations are relatively high in fetal plasma and increase progressively with gestation [228, 229]. The content of fetal pancreatic glucagon at mid-gestation is approximately 6 μg/g, compared with an adult concentration of 2 μg/g. As in the case of insulin, the capacity for glucagon secretion is blunted in the fetus. Hyperglycaemia does not suppress fetal plasma glucagon concentrations in rats, monkeys, or sheep and acute hypoglycaemia does not stimulate glucagon secretion in the rat fetus. Amino acids, which are important secretagogues for insulin and glucagon in the adult, probably have little role in modulating insulin and glucagon secretion in the preterm fetus but infusion of alanine into women at term increases both maternal and cord blood glucagon concentrations, indicating a fetal glucagon response to amino acids in the term fetus. Catecholamines also evoke glucagon release in the near-term ovine fetus [228].

At birth the pancreatic islet cells are functionally immature with regard to their potential to secrete insulin and glucagon. This is most likely due to the relatively stable fetal serum glucose concentrations maintained by placental transfer of maternal glucose. Alternatively, the lack of any enteric signal to the fetal pancreas from feeding via release of incretins may also account for this

stability. The blunted capacity for insulin and glucagon secretion has been related to a deficient capacity of the fetal pancreatic islet cells to generate cAMP or to rapid destruction of cAMP by phosphodiesterase, or both [228]. In the neonatal period there is rapid maturation of glucose responsiveness in term and preterm infants. At birth in the human, there are 200–300 $\times 10^6$ β-cells, approximately one-third of the population present in adulthood. Most of the mass change takes place in the newborn period and is associated with changes in β-cell size rather than number [233]. Subsequently, there is rapid further expansion in terms of cell numbers but the fluctuations in β-cell mass, particularly with pregnancy, are poorly understood.

Fetal energy needs are met by a continuous supply of glucose across the placenta, with little endogenous production. The constant supply of glucose usually precludes the need for endogenous gluconeogenesis and gluconeogenic enzyme expression and function are low in the fetal liver. Fetal glucose uptake is directly related to both the maternal blood glucose concentration and the transplacental gradient, and insulin and glucagon are normally not necessary for substrate metabolism in the fetus [229]. The fetal respiratory quotient is approximately 1, suggesting glucose is the primary energy substrate. Other substrates, such as amino acids and lactate, may also be used in the human as in the sheep fetus and these, together with glucose, are stored as fat and glycogen in preparation for birth [228]. Glycogen storage in the fetus is modulated by fetal glucocorticoids and probably by PL. Near term, fetal insulin becomes increasingly important, increasing fetal glucose uptake and lipogenesis [228, 229]. Insulin receptors are present within numerous fetal tissues at much higher concentrations than in adult tissues, and down-regulation of receptor binding does not occur in the presence of fetal hyperinsulinaemia. Fetal hepatic glucagon receptors, in contrast, are reduced in number and fetal liver is relatively resistant to the glycaemic effects of glucagon [231]. These conditions tend to potentiate the fetal anabolic milieu during the period of rapid growth in the last trimester of gestation.

Pancreatic Regulation of Glucose Homeostasis

Birth severs the placental source of glucose and a term infant experiences an immediate drop in blood glucose concentration during the first 2–4 hours of postnatal life, reaching concentrations of about 2.5 mmol/L (45 g/dL) [234, 235]. Low neonatal blood glucose concentrations occur as part of normal transition to extrauterine life and are usually asymptomatic and transient. The actions of counter-regulatory hormones result in glucose homeostasis by approximately 72 hours of life [234]. Glucagon, released from the pancreatic α-cells, promotes hepatic glycogenolysis and gluconeogenesis. Catecholamines, which markedly increase at the time of parturition, stimulate glucagon and inhibit insulin release [229, 231, 236], thereby contributing to stabilization of blood glucose concentrations. Activity of phosphoenolpyruvate carboxykinase (PEPCK), a gluconeogenic enzyme, also increases during this period.

In the postnatal period plasma, free fatty acids increase due to the effects of catecholamines and thermogenesis. Fatty acid oxidation is central for gluconeogenesis, providing both an energy source to support gluconeogenesis and acetyl coenzyme A (acetyl-CoA) to activate pyruvate carboxylase and hence gluconeogenesis. Furthermore, glucose is spared for utilization by dependent tissues such as brain [219]. Failure of this sequence of physiological changes can lead to hypoglycaemia, which is most common in the first few hours after birth.

Hypoglycaemia secondary to HI can be transient or permanent and is due to the inappropriate secretion of insulin in the presence of low plasma glucose concentrations [237]. Hyperinsulinaemic hypoglycaemia is the most frequent cause of severe, persistent, recurrent hypoglycaemia in neonates and children. Transient HI lasting days is associated with maternal diabetes mellitus, maternal sulphonylurea treatment and glucose infusions during labour. Transient HI lasting for days to months may be due to intrauterine growth retardation (IUGR), perinatal asphyxia or BWS and might require treatment [238]. Permanent HI can histologically be divided into focal and diffuse forms, which are inherited in a sporadic or autosomal manner, respectively. At a molecular level, mutations have been described in 12 genes to date implicated in the insulin secretion pathway [238–240] (see Chapter 16).

Maternal hyperglycaemia also leads to HI and β-cell hyperplasia in the infant. Poorly controlled maternal diabetes mellitus is associated with fetal macrosomia, increased risk of spontaneous miscarriage and fetal malformation. Infants of diabetic mothers are prone to polycythaemia, renal vein thrombosis, hypocalcaemia, respiratory distress syndrome, jaundice, persistent fetal circulation, cardiomyopathy, congenital heart disease and malformations of other organs.

Neonatal diabetes is a form of monogenic diabetes involving severe disruption in β-cell function. It is diagnosed before 6 months and is rare with an incidence of approximately 1:200,000–250,000 [241]. Neonatal diabetes can be permanent, requiring lifelong treatment, or transient. Of the two types, transient neonatal diabetes is more common (60% of cases), and while remission of diabetes before the age of 18 months is seen, or may be so mild that treatment is not required, relapse during adolescence is common. There are many different subtypes of neonatal diabetes and most cases involve isolated diabetes. Many of the known monogenic causes are also

characterized by a variety of syndromic features, reflected in the increasing list of causal genes, alongside an expansion of phenotypic characteristics in syndromic forms (see Chapter 15).

Development of the Parathyroid/Calcitonin System

Parathyroid Embryology

The parathyroid glands originate from the branchial apparatus, which develops during 3–4 wpc. The branchial apparatus is composed of four external branchial clefts of ectodermal origin and five internal branchial pouches of endodermal origin, with branchial arches of mesodermal origin in between. The branchial apparatus undergoes involution to leave derivatives including the parathyroid glands, thyroid gland, thymus, ultimobranchial body, Eustachian tube, middle ear and external auditory canal [242].

The parathyroid glands develop as epithelial thickenings of the dorsal endoderm of the third and fourth branchial pouches. The fourth branchial pouch gives rise to the superior parathyroid glands and the thyroid. The higher third branchial pouch gives rise to the inferior parathyroid glands and thymus. The relative position of the inferior and superior glands, named according to their final location, changes because of the migration of embryological tissues, and parathyroid gland development proceeds in synchrony with thyroid embryogenesis [22, 243].

With the descent of the thymus into the thoracic cavity, the associated inferior parathyroids are also carried caudally until the time of separation from the thymus and hence come to be situated lower in the neck at the lower poles of the thyroid lobe than the superior parathyroids [22, 243]. The fourth pouches encounter the thyroid anlage later and come to rest at the upper poles of the thyroid lobes as the superior parathyroid glands [22, 243]. The parathyroid glands enlarge from <0.1 mm at 12 wpc to 1–2 mm at term. The parathyroid gland contains two cell types, chief cells and oxyphil cells. Chief cells (also known the parathyroid gland principal cells) are predominant and play a critical role in calcium homeostasis. Chief cells produce and secrete parathyroid hormone (PTH) in response to low extracellular calcium concentrations detected by receptors in the cell membrane. Larger oxyphil cells form scattered clusters between chief cells, and their function is unknown. Chief cells spend most of their time inactive due to normal calcium homeostasis. The fifth pouches contribute paired ultimobranchial bodies that are incorporated into the developing thyroid gland as the parafollicular or C cells that secrete calcitonin. The calcitonin content of C cells in the neonatal thyroid gland is as high as 540–2100 mU/g of tissue, concentrations as much as 10 times those observed in the normal adult gland [244]. Both endocrine systems are functional during the second and third trimesters.

Transcription Factors Involved in the Development of Parathyroid Glands

Studies of patients with hypoparathyroidism and use of appropriate mouse models are providing insights into the molecular signalling pathways implicated in the development of parathyroid glands. Several transcription factors, which include members of the homeobox (*Hox*) and paired box (*Pax*) families, have been shown to play important roles in the differentiation of the pharyngeal pouch endoderm into parathyroid cells. Disruption of *Hox15* in mice results in parathyroid aplasia, indicating that this gene is critical for normal parathyroid development. Deletions of genes in mice including members of the *Hox* family (*Hoxa3*, which results in parathyroid agenesis, and *Pbx1*, which encodes a protein that acts as a cofactor of HOX transcription factors) and *Pax* (*Pax 1,3,9)* family, the eyes absent homologue (*Eya*1) and sine oculis homeobox homologue (*Six1*) (Pax1/9-Eya1-Six1 network has been identified to act downstream of *Hoxa3*) result in abnormal development and patterning including parathyroid defects [129]. Such developmental anomalies, however, have not yet been reported in man.

Hypoparathyroidism can occur in isolation, as part of multiple autoimmune endocrinopathies, or as a complex congenital defect. Mutations in *GATA3* (hypoparathyroidism, deafness and renal dysplasia [HDR] syndrome), *AIRE1* (autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy [APECED] syndrome or autoimmune polyglandular syndrome type 1 [APS1]), and *tubulin folding cofactor E* (*TBCE*) (Kenny–Caffey syndrome), have been found in patients with congenital hypoparathyroidism. *TBX1* (a DNA-binding transcriptional factor of the T-box family) and *CRK1* mutations have been associated with 22q11 deletion syndrome [245].

Isolated hypoparathyroidism can be found in association with mutations in *PTH*, *GCMB* (human homologue of the mouse glial cells missing 2 [*Gcm2*], expressed exclusively in the parathyroid glands) and the SRY-related HMG-box gene 3 (*SOX3*) [129]. X-linked recessive hypoparathyroidism has been reported in two multigenerational, related kindreds with a deletion–insertion downstream of *SOX3* likely to exert a position effect on *SOX3* expression. These findings suggest a potential role for *SOX3* gene in parathyroid development.

Fetal Bone Metabolism

In utero, the calcium concentration in the fetus appears to be independent of maternal calcium concentration (Figure 3.12). In animal models, despite maternal

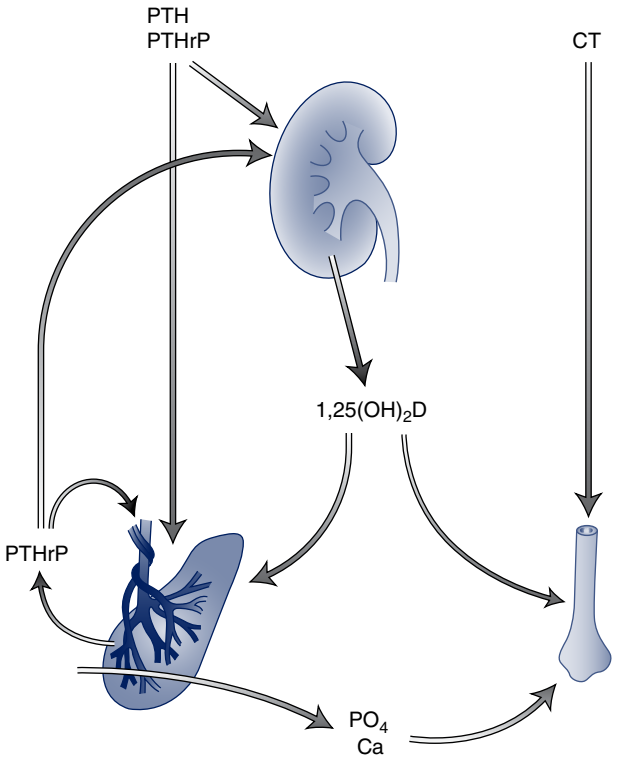

Figure 3.12 Proposed actions of parathyroid hormone (PTH), PTH-related protein (PTHrP) and calcitonin (CT) in the fetus. PTHrP and perhaps PTH from the parathyroid glands and PTHrP from the placenta act on the placenta to promote calcium (Ca) and phosphate (PO_4) transport from the maternal to the fetal circulation to maintain the relative fetal hypercalcemia and the high rate of fetal bone formation during the last half of gestation. PTHrP also acts on the kidney to promote 1-hydroxylation of 25-hydroxycholecalciferol to 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) (calcitriol), which augments placental calcium transport and promotes fetal bone growth. High fetal CT levels tend to promote bone accretion.

hypocalcaemia induced by a calcium-restricted diet, parathyroidectomy, or a vitamin D receptor deletion model, fetuses were reported to be normocalcaemic.

1) **Calcium Transport**

Animal studies and measurements in human preterm and term infants indicate that most calcium is transported to the fetus in the third trimester. Concentrations of total calcium (~2.75–3 mmol/L in the third trimester) and ionized calcium, as well as phosphate and magnesium, are higher in the fetal circulation compared with maternal, demonstrating active transport of calcium across the placenta against a concentration gradient [246, 247]. The majority of calcium is used by the fetus for skeletal mineralization [248]. A three-step model of transplacental calcium transport has been proposed [247,249]:

1) Calcium ion influx from the maternal circulation across the placental membrane via voltage-dependent calcium channels (transient receptor potential cation channel, subfamily V, member 6 [TRPV6], a calcium channel that opens in the maternal-facing basement membrane of the syncytiotrophoblast enabling calcium entry into the cells) at the apical brush-border membrane of the trophoblast cells at the maternal–placental interface.
2) Passage of calcium through trophoblastic cytosol, involving intracellular calcium-binding proteins (calbindin D9K), which transport calcium to the basal membrane.
3) Efflux of calcium ions from cytosol across the basolateral placental membrane, involving a basolateral membrane ATP-dependent calcium pump (PMCA3, at the placental–fetal interface) that transports calcium to the fetal circulation.

2) **Phosphate transport**

Phosphate is also maintained at a higher concentration in the fetal circulation compared with maternal circulation (by 1.5 mg/dL in late gestation) to meet the demands of fetal bone mineralization [250]. The type IIb sodium-dependent inorganic phosphate transporter (NaPi-IIb) is expressed in embryonic visceral and parietal endoderm as well as in labyrinthine cells of the placenta and likely has a role in fetal phosphate homeostasis [249]. Although the fetal concentration of magnesium is also increased compared with the maternal concentration, the gradient is less (~0.12 mg/dL) than those of calcium and phosphate [250]. While the molecular mechanism of transplacental calcium transport has been well studied, little is known about the transport mechanisms of phosphate and magnesium. 25-Hydroxyvitamin D and 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) are transported across the placenta and free vitamin D concentrations in fetal blood are similar to or higher than maternal concentrations [246, 251].

3) **Regulation of calcium homeostasis by PTH/PTHrP**

The chief cells of the parathyroid glands secrete PTH, an 84-amino acid protein, released in response to hypocalcaemia. It is unknown whether the human placenta produces PTH. PTH stimulates bone resorption of calcium, increases renal calcium absorption, and promotes renal synthesis of active $1,25(OH)_2D$, which stimulates calcium uptake from the gut. The fetal sheep has low circulating concentrations of PTH but can increase serum PTH concentrations in response to a fall in serum calcium concentration induced by ethylenediaminetetraacetic acid (EDTA) and can respond promptly to infused calcium with increased serum calcitonin concentrations. In this model, fetal parathyroidectomy decreases placental calcium transport and lowers fetal serum calcium.

In the human fetus, the effect of PTH on placental calcium transport is uncertain; however parathyroid hormone-related protein (PTHrP) is present in fetal tissues and placenta and stimulates calcium transport. PTHrP is produced by many tissues, including the placenta and parathyroids, and acts on PTH receptors. The principal PTH receptor is PTHR1, which has equal affinity for PTH and PTHrP. A second receptor PTHR2 is present in CNS and binds PTH but not PTHrP, whereas PTHrP action in the placenta occurs likely through a distinct receptor that binds PTHrP but not PTH [249, 252].

The PTH concentration in the fetus near the end of gestation is lower (<4.72 pg/mL) than maternal concentrations. In cord blood, the concentration of PTHrP is 15-fold higher than that of PTH at term and plays a critical role in perinatal calcium homeostasis and maintenance of the higher fetal calcium set point [247, 253].

Maternal PTH concentrations are suppressed during pregnancy compared with those in the non-pregnant adult, and fetal PTH concentrations appear to be strongly suppressed. Little PTH is detectable in fetal plasma using immunoassays [254], however although fetal PTH concentrations are very low, fetal mice lacking parathyroid glands or the PTH/PTHrP receptor are hypocalcaemic and have undermineralized skeletons [248, 255, 256]. The principal factor responsible for this bioactivity would appear to be PTHrP rather than PTH.

Knockout of the mouse gene encoding PTH/PTHrP, however, results in hypocalcaemia in the presence of normal or increased placental calcium transport, suggesting that other factors are involved in the maintenance of fetal serum calcium concentrations [246, 257].

PTH-null mice have enlarged parathyroids that are incapable of making PTH, whereas *Gcm2*-null mice lack parathyroids but have PTH that arises from the thymus. *PTH* nulls served as a model of complete absence of PTH, whereas *Gcm2* nulls are a model of severe hypoparathyroidism. The *PTH*-null model demonstrates that PTH contributes to fetal mineral homeostasis because a fetal hypoparathyroid phenotype results with hypocalcaemia, hypomagnesaemia, hyperphosphataemia, low amniotic fluid mineral content and reduced skeletal mineral content in its absence. Although PTHrP acts in concert with PTH to regulate fetal mineral homeostasis and placental calcium transfer, unlike PTH, it does not up-regulate in response to fetal hypocalcaemia.

The fetal parathyroid–placental axis promotes maternal–fetal transfer of bone mineral and accretion of fetal bone mineral. PTHrP also has a major role in fetal bone development and metabolism as well as fetal calcium homeostasis. PTHrP knockout mice display increased ossification of the basal portion of the skull, long bones, vertebral bodies and pelvic bones and mineralization of the normally cartilaginous portions of the ribs and sternum; as a result of the cartilaginous mineralization, the animals die of asphyxiation in the early neonatal period [247, 253, 258, 259]. Animals with combined *PTH* plus *PTHrP* or *PTH/PTHrP receptor* gene ablation have a more severe skeletal chondrodysplasia [256, 260]. Knockout of the mouse calcitonin or vitamin D receptor gene results in postnatal osteosclerosis or osteomalacia, respectively, although the pups appear normal at birth [261]. It seems likely that fetal PTH and presumably PTHrP act on the fetal kidney to stimulate 1α-hydroxylation of 25-hydroxyvitamin D and that $1,25(OH)_2D$ participates in modulating placental calcium transport. $1,25(OH)_2D$ and $24,25(OH)_2D$ also play a role in fetal cartilage growth and bone mineral accretion [262].

4) $1,25(OH)_2D$

During pregnancy, maternal 25OHD crosses the placenta, so the fetal concentrations reach 75–100% of the maternal concentration at term [263]. Fetal $1,25(OH)_2D$ concentrations, however, are lower than that in the mother (<50%) [264]. The maternal concentration of $1,25(OH)_2D$ during pregnancy is two- to threefold higher than that in the non-pregnant state. It is suggested that the maternal kidneys contribute to the abundance of $1,25(OH)_2D$ in the pregnant mother [265]. Nephrectomy in the fetal sheep reduces fetal serum calcium concentrations and this can be prevented by prior administration of $1,25(OH)_2D$. Furthermore, infusion into the sheep fetus of anti-$1,25(OH)_2D$ antibody reduces the placental calcium gradient [246]. The fetal kidney can produce $1,25(OH)_2D$ and the placenta contains $1,25(OH)_2D$ receptors, as well as a vitamin D-dependent calcium-binding protein [259]. It is thought that the synthesis of $1,25(OH)_2D$ in the fetus is suppressed by the high concentrations of calcium and phosphate and low concentrations of PTH.

5) Calcitonin

Calcitonin, produced by the parafollicular (C cells) of the thyroid gland, is involved in helping to regulate concentrations of calcium and phosphate in the blood, opposing the action of PTH. The high concentrations of calcitonin in the fetus, probably resulting from the chronic stimulation by fetal hypercalcaemia, are thought to contribute to the fetal bone mineral accretion [246, 256]. Placental calcitonin production may contribute to the fetal calcitonin concentration *in utero*, but the persistence of high plasma concentrations in the neonate argues for predominant fetal production. Thyroid C cells begin to differentiate at around 10 wpc [266] and calcitonin is detectable at around 13 wpc. Fetal circulating calcitonin concentrations are approximately twofold higher than those in the mother [250, 267, 268]. The trophoblasts of the placenta also produce calcitonin [269]. Since

calcitonin does not cross the placenta in the mouse or rat [270], fetal circulating calcitonin is derived from fetal sources.

6) **Calcium-sensing receptor (CaSR) and FGF23**

The calcium-sensing receptor (CaSR) plays a pivotal role in calcium metabolism by modulating production and secretion of calcium-regulating hormones such as PTH, calcitonin, FGF23 and vitamin D and urinary calcium excretion [271, 272]. The CaSR is present in parathyroid glands, renal tubules, bone and cartilage and other tissues. Magnesium binds to the CaSR and influences PTH secretion. Mutations within the *CaSR* result in either inactivation of the receptor resulting in hypercalcaemia or over-activation of the receptor resulting in hypocalcaemia [273]. Inactivating mutations switch off PTH secretion at a lower calcium concentration and hypercalcaemia ensues. Renal calcium secretion is reduced.

Hypercalcaemic disorders related to inactivating mutations of the *CASR* gene are either heterozygous (autosomal dominant familial benign hypercalcaemia (FBH), still named hypocalciuric hypercalcaemia syndrome type 1) or homozygous (severe neonatal hyperparathyroidism). The differential diagnosis consists of the hypocalciuric hypercalcaemia syndrome type 2 (involving G-protein subunit alpha11 [*GNA11*]) and the hypocalciuric hypercalcaemia syndrome type 3 (involving *AP2S1*, involved in G-protein internalization), hyperparathyroidism, abnormalities of vitamin D metabolism involving *CYP24A1* and *SLC34A1* genes, and reduced GFR.

Hypocalcaemic disorders, which are more rare, are related to heterozygous activating mutations of the *CASR* gene (type 1), consisting of autosomal dominant hypocalcaemia (ADH), sometimes with a presentation of pseudo-Bartter's syndrome. The differential diagnosis consists of the hypercalciuric hypocalcaemia syndrome type 2, involving *GNA11* and other hypoparathyroidism aetiologies.

Neonatal Disorders of Calcium Homeostasis

Some conditions affecting calcium homeostasis are particularly important in the neonatal period. When a neonate presents with abnormal calcium concentrations, assessment of calcium, albumin, phosphate, creatinine, alkaline phosphatase, vitamin D and urine Ca–creatinine ratio and phosphate reabsorption, in both the child and parents, will help formulate the underlying diagnosis. Hypocalcaemia resulting from fetal and maternal vitamin D deficiency is relatively common. Occasionally, severe neonatal vitamin D deficiency can be associated with a dilated cardiomyopathy, which is reversible [274, 275]. Asphyxia can result in subcutaneous fat necrosis, which is due to release of $1,25(OH)_2D$ from macrophages and leads to hypercalcaemia. Hyperhydration, steroid therapy, low-calcium milk and occasionally bisphosphonate treatment may be needed to reduce calcium concentrations.

FBH and familial hypocalciuric hypercalcaemia (FHH) can present in neonates with mild to moderately elevated calcium concentrations, high normal (non-suppressed) PTH and a low urine calcium–creatinine ratio [276]. FHH may also present as neonatal hyperparathyroidism leading to bone disease and variable symptoms of hypercalcaemia. This occurs especially if an affected infant has inherited the inactivating *CaSR* mutation from the father while their mother is normal. The fetus will consider the mother as hypocalcaemic and therefore overproduce PTH. Cinacalcet therapy may help bind calcium and settle the hyperparathyroidism but parathyroidectomy is occasionally necessary. Hypo- and hypercalcaemic disorders may also be due to mutations in *GNA11*, *AP2S1* and calcium channels *TRPV5* and *TRPV6* [277, 278].

FGF23 is the principal hormone that regulates phosphate transport. It is primarily produced and secreted into the circulation by osteocytes and, after its activation through glycosylation by GALNT3, acts through FGFR1c and FGFR4 on renal tubules to increase phosphate excretion through the sodium-phosphate exchanger (NaPi-IIC, SLC34A3) [279]. Alpha-Klotho acts as a cofactor for FGF23 to bind to FGFR1c, increasing specificity of FGFR1c for FGF23. FGF23 is inactivated by cleavage by subtilisin/furin-like enzyme. PHEX (phosphate-regulating gene with homologies to endopeptidases on the X chromosome) regulates cleavage of FGF23 and *PHEX* mutations therefore render FGF23 constitutively active. Besides increasing renal tubular phosphate excretion, FGF23 inhibits 1-alpha hydroxylase, thus reducing 1,25-vitamin D activity. Loss of FGF23 or components of the FGF23 network (GALNT3, FGF23, alpha-Klotho) causes hyperphosphataemia, extra-skeletal calcifications and early mortality; excess FGF23 or pathway components (FGF23, PHEX, SLC34A3, subtilisins [PCSKs], phosphate transporters NPT2a and NPT2c) cause hypophosphataemia with rickets or osteomalacia [280]. However, FGF23 may not be important during fetal development. In a study by Ma et al. [281], FGF23 deficiency (*Fgf23* null) and *FGF23* excess (*Phex* null) did not alter fetal phosphorus or skeletal parameters. Although FGF23 is present in the fetal circulation at concentrations that may equal adult values and there is robust expression of *FGF23* target genes in placenta and fetal kidneys, FGF23 itself may not be an important regulator of fetal phosphorus metabolism.

Endocrine Regulation of Fetal Growth

Fetal growth is regulated by complex interactions between maternal, placental and fetal factors, including

nutritional, environmental and hormonal. The hormones most crucial for postnatal growth (T_4, GH and gonadal steroids) have a limited role *in utero* [282]. PLs play a minimal role as PLs may promote early embryonic growth and may stimulate IGF and insulin production [282]. Insulin and the IGF system play important roles in regulating normal fetal growth. IGF1 and IGF2 are produced by the placenta and may exert autocrine–paracrine actions on placental growth. IGF1 and IGF2 and their receptors are widely expressed in fetal tissues and play a critical role in modulating fetal growth [283, 284].

The IGF system

The IGF system in mammals comprises two ligands (IGF1 and IGF2), two receptors (IGF1R and IGF2R) and six binding proteins (IGFBPs). Autocrine and paracrine roles for the IGFs in uterine and placental tissues are postulated. Both IGF1 and IGF2 are expressed in fetal tissues from the earliest stage of pre-implantation to term. IGF2 primarily supports embryonic growth, with IGF1 playing an increasingly important role as pregnancy progresses. IGF1 is a mitogen for endometrial stromal cells and endometrial expression of *IGF1* mRNA and IGF1 is high during early embryogenesis in the sow [285]. Placental tissue also contains IGF1 and IGF2 and IGF1 receptors [59]. Embryonic tissues produce IGF1 and insulin during the pre-pancreatic stage of mouse development; both factors stimulate growth of embryonic mouse cells [286]. *IGF2* is genomically imprinted and paternally expressed in the fetus and placenta. The mature IGF2 protein is generated from the biologically inactive pro-IGF2 by the action of proprotein convertase 4. Previous studies have shown a role of IGF2 in determining placental nutrient supply and, hence, fetal growth [287]. In mutant mice lacking the placental-specific *IGF2* transcript, growth of the placenta is altered from early gestation but fetal growth is normal until late gestation, suggesting functional adaptation of the placenta to meet fetal demand. It is believed that this adaptation may be mediated by the altered expression of the placental transporters, namely, glucose transporter 3 (*GLUT3*), also known as solute carrier family 2, facilitated glucose transporter member 3 (*Slc2a3*), and *Slc38a4/SNAT4* (an imprinted member of the System A amino acid transporter) [288]. GLUT3 and Slc38a4 appear to be a central link between placental supply and fetal demand.

Studies of transgenic mice with null mutations of *IGF1*, *IGF2* or the *IGF1R* have defined the role of these somatomedins. The birth weight of the embryos lacking IGF1 or IGF2 is only 60% that of control mice, and when both *IGF1* and *IGF2* are inactive, birth weight is reduced by a further 30%. Knockout of individual IGF binding proteins has little effect on fetal growth [55]. In mice lacking the *IGF1R*, birth weights are on average 45% those of controls [55]. *IGF2*-deficient mice also manifest IUGR in association with a small placenta. They have near-normal postnatal growth but delayed bone development [55]. IGF2 receptor knockout fetal mice are 30% overweight, suggesting a negative growth-modulating effect of this receptor. Normal growth is seen in fetuses with both IGF1 receptor (*IGF1R*) and IGF2 receptor (*IGF2R*) knockout and this is due to IGF1 signalling via the insulin receptor; combined *IGF1*, *IGF2* and insulin receptor knockout results in severe IUGR and fetal death. The IGF system appears to play a crucial role in fetal growth; however naturally occurring mutations in the IGF system are very rare in humans. Human mutations in *IGF1* or *IGF1R* are associated with IUGR [289, 290], suggesting that IGF1 signalling contributes significantly to fetal growth. There is an association between umbilical IGF1 concentration and birth weight in humans as maternal smoking reduces both umbilical IGF1 and birth weight [291, 292]. Additionally, work in mice suggests that IGF1 and IGF1R are required for late gestational lung maturation [293], which is important for transition to extrauterine life.

Imprinting and Fetal Growth

Genomic imprinting is important in fetal growth, as demonstrated by the phenotypes observed in uniparental disomy in mice and humans. Paternal disomies appear to be growth promoting and maternal disomies growth inhibiting. A number of growth-related genes, including those regulating the IGF system, are imprinted. The *IGF2* gene, for example, is paternally expressed, whereas the *IGF2R* gene is maternally expressed. The paternally expressed *IGF2* gene is located within the imprinted 11p15 region. Studies of several mouse models have demonstrated this region plays a key function in fetal growth. The 11p15 region includes other paternally expressed (such as *IGF2* and *KCNQ1OT1*) and maternally expressed (such as *CDKN1C* and *H19*) genes. Given their role in fetal growth, imprinted genes within the 11p15 region are also good candidates for the aetiology of diseases that involve IUGR. Hypomethylation of the 11p15 imprinted region has been associated with the phenotype of Silver–Russell syndrome (SRS), a condition in which severe IUGR and reduced postnatal growth are associated with dysmorphic facial features and body asymmetry [294]. This results in the relaxation of imprinting and biallelic expression of *H19* and down-regulation of *IGF2*. Additionally, abnormal processing of IGF2 by proprotein convertase 4 in the placenta has been implicated in the aetiology of fetal growth restriction [295]. Pregnant women carrying fetuses with IUGR had higher concentrations of pro-IGF2 compared with controls. Recently, a genomic mutation of the human *IGF2* gene was described in four

individuals from one family who exhibited severe intrauterine and postnatal growth retardation and signs of SRS [296]. The defect was a nonsense mutation with a predicted severe truncation of the pre-pro-IGF2; the disorder was paternally inherited and affected children had diminished IGF2 serum concentrations.

IUGR is the main feature of mouse knockout models lacking *Igf2*. In humans severe IUGR and atypical diabetes mellitus secondary to insulin resistance have been reported in association with disruption of regulation of the *IGF2* gene [297]. On the other hand, fetal overgrowth is caused by caused by genetic or epigenetic changes in 11p15, leading to the down-regulation of maternally expressed and/or the up-regulation of paternally expressed genes. Overexpression of *IGF2* as a result of loss of imprinting associated with uniparental paternal disomy, *CDKN1C* gene loss of function, alteration in the *KCNQ1OT1* differentially methylated region (DMR), or microdeletions in the human *H19* DMR is associated with overgrowth in the form of BWS [298]. As described previously, mutations in the PCNA-binding domain of *CDKN1C* have been shown to cause IMAGe syndrome [97]. Missense mutations in the PCNA-binding domain have been shown to have an inhibitory effect on growth and differentiation *in vivo*, via loss of binding of PCNA to CDKN1C. The contrast between BWS and IMAGe mutations in *CDKN1C* highlights the dual and opposing effects of specific *CDKN1C* mutations.

IGFs and IGFBPs

IGFs circulate with IGFBPs prenatally as well as postnatally and IGFBPs are present as early as 5 weeks' gestation [55]. High concentrations of circulating IGFBP1 are associated with IUGR in the mouse, as is overexpression of fetal IGFBP1 in the human [299, 300]. IGFBP4, expressed in the maternal decidua and cleaved by its metalloproteinase PAPP-A (pregnancy-associated plasma protein A), inhibits IGF action. High maternal concentrations of IGFBP4 are linked to fetal growth restriction [295].

During fetal and postnatal life, plasma concentrations of IGFs are relatively high compared with tissue concentrations. In the fetus, IGF2 concentrations are 5–6 times higher than those of IGF1 (in contrast to these concentrations in children and adults). Concentrations of both IGF1 and IGF2 increase progressively throughout gestation [301] and fetal concentrations at term are 30–50% of the adult concentrations. The high concentrations of IGF2 in fetal rat serum, the high concentrations of IGF2 mRNA in fetal tissues and the presence of a truncated form of IGF1 in human fetal brain tissue suggest unique developmental actions of these peptides.

PAPP-A2 regulates IGFBPs and is hypothesized to increase IGF1 bioactivity by specific proteolytic cleavage of IGFBP3 and IGFBP5. Mutations in *PAPPA-A2* have been shown to cause short stature due to low IGF1 availability as part of a novel syndrome of growth failure [302]. The two mutations described were shown to cause a complete absence of PAPP-A2 proteolytic activity, a significant increase in bound IGF1 and decreased free IGF1 concentrations, highlighting the critical role of PAPP-A2 in releasing IGF1 from its BPs.

In most studies, cord blood IGF1 concentrations correlate with birth size. Despite the fetal growth-enhancing effects of IGF2, blood concentrations are only weakly related to size at birth, largely because of the inhibiting effect of soluble IGF2R [284] but also because IGF2 appears to exert most of its growth effects in the earlier part of gestation. IGF receptors have been identified as early as 5 weeks' gestation and are widespread in fetal tissues [303].

The control of IGF production differs in fetal and postnatal life. GH receptors are present but receptors for PL predominate in fetal tissue GH [40], which stimulates IGF1 production postnatally, has a limited role in fetal IGF production [283]. GH does however play a minor role in fetal growth, as reflected in the low IGF concentrations and slight reduction in birth weight and length in infants with GH resistance (Laron dwarfism) and GH deficiency [304]. PL stimulates IGF1 production and augments amino acid transport and DNA synthesis in human fetal fibroblasts and muscle cells [40]. IGF1 and IGF2 concentrations are reduced in fetuses of protein-starved pregnant rats and the low IGF2 concentrations are reversed by PL [305]. Thyroidectomy of third-trimester sheep fetus impairs skeletal muscle growth in association with a decrease in muscle GH receptor mRNA and *IGF1* mRNA without an effect on IGF2 concentrations [306]. Glucocorticoids can inhibit fetal growth, presumably by inhibiting *IGF* gene transcription, but may also affect growth plate chondrocytes directly [306]. Nutrition is the major factor modulating IGF production in the fetus. IGF concentrations fall in suckling rats deprived of milk and IGF1 and IGF2 concentrations are reduced in fetuses of protein-starved pregnant rats and sheep with placental insufficiency [54, 55]. Recent work suggests that light stimuli alter circulating and brain IGF1 concentrations and control neuronal migration through increased IGF1 signalling [307]. Weekly intra-amniotic IGF1 injections increase fetal growth of growth-restricted sheep [308]. These data support the view that IGFs are important in fetal growth and that in the fetus they are regulated, at least in part, by PL and by nutritional substrate derived transplacentally.

Insulin

Insulin plays a role in fetal growth and low birth weight has been reported to be associated with impaired insulin

secretion and insulin resistance. Infants of mothers with diabetes may have hyperinsulinaemia associated with increased birth weight [309]. Body fat accounts for most of this increased weight, and some organomegaly may occur, with little increase in body length. Infants with hyperinsulinaemia due to congenital HI or BWS may also have increased somatic growth *in utero*, and are born large for gestational age, primarily due to increased lipogenesis mediated by insulin or IGF1 receptors. Conversely, pancreatic agenesis results in a small human fetus with decreased muscle bulk and little or no adipose tissue [309].

Insulin receptors are increased in fetal cells and are resistant to down-regulation. Mice with insulin or insulin receptor mutations are found to have a 10% decrease in birth weight and early neonatal death with hyperglycaemia and ketosis [55]. Insulin receptor mutations in humans lead to severe IUGR and limited postnatal weight gain [55]. In contrast to mice, the human fetus during the latter half of gestation has a significant increase in adipose mass and adipose tissue is highly sensitive to insulin. Treatment with IGF1 improves the clinical condition to an extent [310].

Epidermal Growth Factor (EGF)/transforming Growth Factor (TGF) System

During pregnancy, circulating maternal concentrations of EGF are increased and sustained throughout gestation. EGF is a 6 kD peptide product of a large 1207-amino acid precursor molecule and acts through a 170 kD membrane receptor glycoprotein. This has intrinsic tyrosine kinase activity like the IGF receptor and tyrosine kinase-mediated autophosphorylation is a critical event in EGF signal transduction. EGF has mitogenic roles in most organs within the body; thus it is unsurprising that EGF also regulates fetal growth and development. The EGF family comprises 14 different ligands including TGF-α, amphiregulin, heparin-binding EGF, betacellulin and neuregulins [311, 312]. EGF exerts its effects by binding to its receptor EGFR (also known as the erythroblastic leukaemia viral oncogene homologue [ErbB] 1) to stimulate intrinsic tyrosine phosphorylation activity and subsequent activation of pro-mitogenic signalling cascades. Three additional receptors are referred to as ErbB2, ErbB3 and ErbB4 in animals; the human receptors are called human EGF receptor 2 (HER2), HER3 and HER4 [312]. TGF-α, which has 35% amino acid homology with murine EGF and 44% homology with human EGF, also acts through the EGF receptor system [311, 312].

EGF and *pre-pro-EGF* mRNA levels are absent or low in the fetal mouse and remain low in murine tissues during the early neonatal period [313]. EGF, *pre-pro-EGF* mRNA and EGF receptors are present in most tissues in the postnatal rodent. The EGF receptor knockout mouse demonstrates epithelial immaturity and multi-organ failure with early death [313]. Tissue concentrations of both EGF and *EGF* mRNA increase in the mouse during the first 2 months of postnatal life, and it is during this time that most hormone-stimulated growth and development occur. Concentrations of EGF in the mouse salivary glands increase several thousand-fold between 3 weeks and 3 months of age. Mouse urinary levels increase 200-fold and kidney concentrations increase 10-fold between 1 week and 2 months of age. EGF concentrations in mouse ocular tissues increase 100-fold during the first week of life [312]. Liver EGF concentrations increase more slowly, correlating with serum concentrations in the developing mouse [312]. In rodents and sheep, EGF provokes precocious eyelid opening and tooth eruption in neonatal animals, stimulates lung maturation, promotes palatal development in organ culture, stimulates gastrointestinal maturation, evokes secretion of pituitary hormones including GH, PRL and ACTH and stimulates secretion of hCG and lactogen by the placenta [312, 313].

Fetal mouse and human tissues have high concentrations of TGF-α [311, 314, 315]. Immunoreactive TGF-α concentrations are measurable at high levels in fetal/neonatal rodent lung, brain, liver and kidney tissues and the ontogenic pattern of TGF-α is tissue specific [314]. Both EGF and TGF-α compete for binding to the EGF receptor and accelerate eye opening and tooth eruption in the neonatal rodent [312].

Considerable evidence suggests a role for the EGF family of growth factors in mammalian CNS development [316]. EGF, TGF-α, neuregulins and the EGF receptors are widely distributed in the nervous system [312, 317–320]. EGF promotes proliferation of astroglial cells, acts as an astroglial differentiation factor and enhances survival and outgrowth of selected neuronal cells [317, 318]. Transgenic mice with a deficiency of neuregulin, ErbB2, ErbB3, or ErbB4 die *in utero* with cardiac anomalies and developmental anomalies of the hindbrain, midbrain and ventral forebrain [319, 320].

EGF also plays an important role in rodent pregnancy. Maternal salivary gland and plasma EGF concentrations in the mouse increase up to fivefold during pregnancy [321]. Removal of salivary glands prevents the increase in plasma EGF and reduces the number of mice completing term pregnancy by 50%, decreases the percentage of live pups and decreases the crown-rump length of fetuses delivered [321]. Administration of EGF antiserum to pregnant mice without salivary glands further increases the abortion rate, whereas administration of EGF improves pregnancy outcome [321]. Because maternal EGF is too large a molecule to cross the placental barrier, an effect on maternal metabolism or on the placenta is likely [321]. The placenta is richly endowed with EGF

receptors and placental tissue binds and degrades EGF to constituent amino acids [312]. TGF-α is also produced by the maternal decidua in rodents and stimulates proliferation of decidual tissue and decidual PRL production.

Other Growth Factors and Signalling Pathways Involved in Fetal Growth

Additional growth factors involved in fetal growth and development include hematopoietic growth factors, platelet-derived growth factors (PDGFs), fibroblast growth factors (FGFs), vascular endothelial growth factor (VEGF) and members of the TGF-β family [322]. The TGF-β superfamily of extracellular growth factors comprises more than 35 members, including TGF-β, the BMPs, growth and differentiation factors, activins, inhibins, Müllerian-inhibiting substance and Nodal and Lefty proteins [323]. These ligands activate 12 transmembrane serine/threonine kinase receptors expressed in a variety of tissues. The family is critical for early embryonic development, left–right asymmetry, heart and vascular system development, craniofacial development, nervous system development and skeletal morphogenesis and plays an important role in body composition and growth.

Hematopoietic growth factors are also active in the fetus during development; erythropoietin in fetal sheep is produced by the liver rather than the kidney and erythropoietin gene expression in fetal sheep is regulated by glucocorticoids [324]. A switch to renal production occurs after parturition [325]. Postnatally, THs, testosterone and hypoxia modulate erythropoietin production.

The PDGFs A–C and their receptors PDGFR-α and PDGFR-β have been shown to promote cellular responses such as proliferation, survival, and migration [326]. *Pdgfa* gene inactivation in mice leads to defects in lung, skin, intestine, testes and brain, resulting in early postnatal death [326]. In mice, deletion of the gene encoding Pdgfb or Pdgfr-β results in multiple defects in placental development, including decreased trophoblast proliferation, while an activating mutation in Pdgfr-β induces hyperproliferation in the chorionic plate. Although reports of the role of PDGF in regulating human fetal growth are limited, maternal serum PDGFB concentration has been reported to be enhanced in mothers suffering with gestational diabetes with macrosomic babies, and it has been reported that placental concentrations of PDGFRα are low in the growth-restricted fetus [326].

The FGF family comprises 18 members, FGFs 1–10 and FGFs 16–23. Not all members have the potential to signal, but those that do interact with four tyrosine protein kinase receptors (FGFR1–FGFR4) to stimulate mitogenesis, differentiation, and cell migration, with diverse effects on development, angiogenesis, wound healing and other biological systems [327, 328]. Several receptor isoforms are products of alternative RNA splicing [311]. Targeted disruptions of *Fgf* and *Fgfr* genes in mice have defined their critical roles in development [327]. Fgfr1-deficient mice display severe IUGR and knockout of the *Fgfr1* gene leads to early fetal death. Studies in human pregnancy reveal that maternal and cord serum concentrations of FGF2 positively correlate with fetal weight and the effect on fetal growth was also accompanied by alterations in placental growth, suggesting that FGF2 may exert its effects by influencing placental development. Fgf3-deficient mice show tail and inner ear defects. Knockout of the *Fgf4* gene is lethal, leading to early death. *Fgf10* knockout mice die at birth because of pulmonary agenesis. Deficiency of Fgf4, Fgf8, Fgf9, Fgf10, or Fgf17 is associated with limb deformities. Fgf8 deficiency leads to abnormal left–right axis patterning. In mice, *Fgfr3* knockout results in chondrocyte hypertrophy and increased bone length [311]. In humans, a variety of gain-of-function *FGFR* mutations are associated with chondrodysplasias and craniosynostosis syndromes [311]. Loss-of-function mutations in both *FGFR1* and *FGF8* are associated with Kallmann syndrome. *FGF8* mutations are also associated with holoprosencephaly [329–331]. FGF, like EGF, stimulates the production of hCG from a choriocarcinoma cell line [328]. These observations and the fact that the placenta contains FGF, TGF-*α*, TGF-*β*, IGF1 and IGF2 suggest that the placenta plays an important role in modulating fetal growth.

Wnt signalling, Notch signalling, BMP signalling and hedgehog signalling play major roles in embryogenesis and fetal organ growth and development. These signalling pathways are also involved in bone development and growth and thus have a major effect on fetal size [332].

Neutralization of Hormone Activity in the Fetus

During pregnancy, endocrine and metabolic systems are programmed to maintain stability of the fetal milieu in the face of a changing external environment. Fetal growth and development is promoted by growth factors and a constant placental substrate supply, which maintain anabolism. Production of catabolic and thermogenic hormones is attenuated, as are the effects of the hormones altering metabolic substrate supply and distribution (Table 3.4).

Limitation of Hormone Secretion

Functional capacity of the human fetal pancreas occurs during the second trimester; however insulin secretion is limited until the postnatal period [228, 289]. Active glucagon secretion is also constrained although concentrations in the fetal circulation are relatively high. Insulin

Table 3.4 Neutralization of hormone actions in the fetus.

Production of inactive metabolites	
Active hormone	**Inactive metabolites**
Cortisol	Cortisone
Thyroxine (T_4)	rT_3, T_4S, rT_3S
Triiodothyronine (T_3)	T_3S, T_2
Delayed expression or neutralization of receptors	
Active hormone	**Receptor**
Growth hormone (GH)	GHR
Thyroid hormone	TRα, TRβ
Catecholamines	β-AR
Oestrogens	ERα, ERβ
Glucagon	GR
Limited hormone secretion	
Active hormone	**Secretory cell**
Insulin	Islet cell β
Glucagon	Islet cell α

AR, adrenergic receptor; T_2, diiodothyronine; rT_3, reverse T_3; T_4S, T_4 sulphate.

secretion can occur acutely following fetal infusions of arginine, leucine or tolbutamide [229, 232]. Chronic hyperglycaemia, as seen in some pregnancies complicated by maternal diabetes, results in fetal islet cell hyperplasia and insulin secretion. It is not until the neonatal period that islet cell responsiveness to glucose develops. It is unclear if this is secondary to stability of the blood glucose profile *in utero* or due to a temporally regulated maturation process of cells secreting insulin and glucagon.

Production of Inactive Hormone Metabolites

The majority of maternally produced cortisol crossing the placenta, as well as cortisol produced by the fetus, is inactivated to cortisone by the enzyme HSD11B2 in fetal tissues and placenta. Placental HSD11B2 activity increases during the second half of pregnancy under the control of placental oestrogens and enzyme activity near term is high [59, 111]. The conversion of cortisone to cortisol is limited during the majority of fetal life. During mid-gestation however, placental HSD11B2 activity is low and some cortisol is transferred to the fetus. Concentrations of cortisone in fetal plasma are three- to fourfold greater than cortisol until after 30 weeks' gestation (see Figure 3.5). This helps preserve the anabolic and growth-promoting milieu of the fetus. After 30 weeks' gestation, the ratio of cortisol to cortisone in fetal tissues and plasma increases due to increased fetal secretion and decreased conversion of cortisol to cortisone within the placenta and fetal tissues [111]. Cortisol has an important maturational action on several fetal tissues at term and plays an important role in the initiation of parturition.

Delayed Expression or Neutralization of Receptor Response

Fetal TH metabolism is characterized by conversion of active THs to inactive metabolites and for most of gestation, T4 is metabolized primarily to rT3 and a variety of sulphated THs that are biologically inactive. This is dependent on the activity of deiodinase enzymes, which are developmentally regulated in specific tissues, on the process of sulphation, and on the limited receptor and post-receptor responsiveness to TH in selected tissues [122, 333]. The D3 monodeiodinase enzyme catalyses inner-ring deiodination of T4 to transcriptionally inactive rT3 and deiodination of T3 to inactive T2. This enzyme is highly expressed in the uterus, placenta, and amniotic membrane, where it has an important role in regulating placental transfer of maternal THs to the fetus [122, 333]. Another important process *in utero* is sulphation whereby about 80% of T4 produced by the thyroid gland is metabolized to biologically inactive sulphated forms, such as T4S, T3S, and rT3S [122, 333]. THs are sulphated by sulphotransferase, primarily in the fetal liver, but also in the kidneys, brain, and intestines. The fetal sheep liver and kidney, in contrast to the adult liver and kidney, manifest low concentrations of D1 outer-ring monodeiodinase activity, so conversion of T_4 to active T_3 is limited and large amounts of inactive iodothyronine sulphoconjugates accumulate [122, 334]. Consequently plasma T_3 concentrations in the fetus remain low until the last few weeks of gestation when there are developmental changes in deiodinase activity (see Figure 3.7). Selected fetal tissues (brain, BAT) have active D2 outer-ring monodeiodinase activities that contribute to local tissue T_3 concentrations; local T_3 is important in development, particularly in the hypothyroid fetus [122, 335]. Near term and in the neonatal period in the human fetus, the dramatic increase in plasma T_3 concentrations, and presumably in T_3 production, heralds the onset of TH actions on growth and development and on metabolism (see Figure 3.7).

TH bioactivity is also determined by the expression of transporters and intracellular TRs in the target tissues and post-receptor binding pathways. Delayed TH responsiveness is evident, and in addition to developmental programming of iodothyronine monodeiodinase expression, gene expression programming via unliganded TRs or TR-interacting corepressors probably plays a role. The various TRα (THRA) and TRβ (THRB) isoforms are expressed in the fetus in a tissue-specific manner and often at gestational ages earlier than the

appearance of THs in the fetal circulation, suggesting that for some species, maternal THs may contribute to the control of early embryonic growth and development, before the onset of fetal TH activity. Developmental changes in TR binding in the fetal brain, lung, skeletal muscle, liver, and heart are evident as term approaches and are species specific. Fetal ovine liver and kidney thermogenesis is unresponsive to exogenous T_3 during the third trimester and TH responsiveness in a number of tissues (cardiac, hepatic, renal and skin) develops only during the perinatal period [336]. In fetal pigs, there are decreases in TRα expression in skeletal muscle and increases in TRβ abundance in the heart and skeletal muscle at birth. In both species, the gestational changes in fetal tissue TR abundance closely parallel plasma cortisol concentrations; however the effect of the late third-trimester cortisol surge on the expression of TH transporters and receptors *in utero* remains unknown. In rodent species, in which development at birth is comparable with human fetal development at mid-gestation, pituitary GH concentrations become responsive to TH only during the first weeks of extrauterine life [337]. Mouse submandibular gland EGF and NGF concentrations become responsive to TH during the second week of life, as do urine and kidney EGF concentrations and hepatic EGF receptor levels [338, 339]. Mouse skin EGF levels and EGF receptors are responsive during the first neonatal week [340, 341]. Therefore, despite the presence of nuclear T_3 receptors in significant concentrations in developing rat and sheep fetuses, many TH actions in these species are delayed [342].

Similarly, high circulating concentrations of fetal GH are seen; however its effects are limited. Fetal growth is only partially dependent on GH and in fact the GH-deficient fetus has little or no IUGR [40, 304]. The paucity of fetal GH effects is due to delayed maturation of GH receptors or post-receptor mechanisms. In animals such as sheep, hepatic GH receptor binding appears only during the neonatal period [40]. Receptor deficiency may also be a factor in the limited PRL bioactivity in the fetus near term [40].

Very little is known about fetal hormone responsiveness in other systems. β-Adrenergic receptor binding in heart and lung of the ovine fetus is unresponsive to T_3 late in the third trimester but increases in response to T_3 in the neonatal period [22, 336]. Premature lambs have an amplified plasma catecholamine surge at birth but only a slight increase in plasma free fatty acid concentrations, indicating reduced catecholamine responsiveness [343]. The high concentrations of progesterone and oestrogens in fetal blood also appear to have restricted effects in the fetus. Progesterone receptors are present in low concentration in fetal guinea pig kidney, lung and uterus at mid-gestation and increase progressively until term [344]. ERs appear in neonatal rat uterus, oviduct, cervix and vagina during the first 10 days of extrauterine life and both *ERα* and *ERβ* mRNAs are present in human fetal tissues during the second trimester [186, 345]. Mild breast enlargement can occur in the human neonate at birth and vaginal oestrogenization may be evident in female infants. Oestrogen effects otherwise appear to be limited (see Table 3.4).

Plasticity of Fetal Endocrine Systems

The extensive networks linking maternal–placental–fetal endocrine interactions and the apparent plasticity of developing endocrine and metabolic systems facilitate endocrine system programming. The concept of plasticity of fetal endocrine systems evolved from experiments in several species indicating that hormonal programming occurs during a critical fetal or perinatal period of development. In the female rodent, transient androgen administration in the neonatal period has several effects: (1) masculinization of the pattern of hypothalamic control of GnRH secretion and pituitary gonadotropin secretion, adult behaviour and adult sexual activity, (2) permanently altering the pattern of GH secretion, (3) increasing longitudinal bone growth and body weight and (4) masculinization of the pattern of hepatic steroid metabolism [346, 347]. In sheep, antenatal androgen administration can alter the timing of puberty; the higher the dose of prenatal testosterone, the earlier the initiation of the pubertal LH surge [348]. Oestrogen administration to pregnant rats during the third trimester produces cryptorchid male progeny and may permanently suppress adult male spermatogenesis [349]. Transient levothyroxine administration to neonatal rodents leads to growth retardation, delayed puberty, decreased adult pituitary weight, decreased pituitary TRH concentrations, low serum TSH concentration and decreased TSH responsiveness to propylthiouracil challenge [350, 351]. Administration of insulin to neonatal rats produces permanent adjustment of glucose tolerance [352]. A single dose of vasopressin to the neonatal rat permanently enhances the adult response to vasopressin [353]. Rat fetal exposure to high maternal glucocorticoid concentrations inhibits fetal growth and leads to subsequent hypertension in offspring [13]. Furthermore, longitudinal observations indicate that permanent programming can be transmitted to later generations [15, 352]. The concept of fetal programming was extended with the observation of associations between fetal and neonatal/childhood health indicators (e.g. birth size, infant mortality) and adult diseases. This concept, developed in the 1980s and known as the Barker hypothesis [354], has evolved and there is now extensive documentation, for example, of the association of IUGR with

an increased risk of later hypertension, insulin resistance, diabetes and cardiovascular and coronary heart disease [355–362]. Fetal programming involves complex interactions between environmental, epigenetic and genetic factors, neuroendocrine, hormonal receptor and metabolic alterations involving the placenta and fetus. Epigenetic effects include genetic imprinting. Imprinted genes are a class of genes in placental mammals whose expression depends on the parental origin; they are expressed only from the paternal or the maternal gene copy. To date, more than 150 human genes have been shown to be imprinted [363]. Four classes of molecular changes are described in most imprinted disorders: (1) uniparental disomy, (2) chromosomal imbalances, (3) aberrant methylation (epigenetic mutation) and (4) genomic point mutations in imprinted genes. These all alter expression of imprinted genes but it is the parental allele affected by the mutation that determines phenotype.

An increasing range of epigenetic effects and corresponding clinical syndromes are being identified. So far, genomic point mutations in imprinted genes have been reported only for BWS, SRS and Angelman syndromes, precocious puberty and pseudohypoparathyroidism (PHP). Imprinting is controlled epigenetically (by factors such as nutrition) via DNA methylation, post-translational histone modification, chromatin structure and non-coding RNAs. Imprinted loci often comprise several genes under epigenetic regulation leading to stage- and tissue-specific transcriptional activity in cells with identical DNA sequences. In the majority of imprinted disorders, only the disease-specific loci are affected but an increasing number have been reported to exhibit multilocus methylation imprinting disturbance (MLID) [364], the mechanism of which is at present unknown.

Many of the imprinted genes regulate fetal growth [365]. Paternally expressed imprinted genes generally enhance growth and maternally expressed genes tend to suppress fetal growth. Knockout of paternally expressed genes for *IGF2*, *PEG1*, *PEG2* and insulin result in IUGR, whereas knockout of the maternal genes *H19* and *IGF2R* or overexpression of *IGF2* results in fetal overgrowth [366]. More recently, the identification of an *IGF2* mutation in patients with growth restriction indicates that IGF2 mediates not only prenatal growth but also contributes to postnatal growth and has pleiotropic effects [296]. A role of *IGF2* mutations in both overgrowth and growth restriction phenotypes is conceivable, as has been shown for functionally opposing mutations in *CDKN1C* in 11p15.5 [367]. A loss of DNA methylation (LOM) at the *H19/IGF2* domain has been identified in over 50% of patients with SRS [368]. In contrast, gain of methylation at this domain is found in 10% of patients with BWS. Other genetic alterations including modification of tandem repeats in the insulin gene have been described [369].

Other examples of fetal programming include the historical observation that diethylstilboestrol administration to pregnant women increased the prevalence of vaginal adenocarcinoma in female offspring during the second and third decades of life [360]. It was later shown that prenatal or neonatal diethylstilboestrol exposure in hamsters and mice disrupts normal uterine development by affecting the genetic pathways programming uterine differentiation and results in hyperplastic and neoplastic uterine lesions [370, 371].

Excessive androgen exposure during fetal life has been associated with later polycystic ovary syndrome [360]. Hormonal programming is also demonstrable in cell lines and in unicellular organisms, in which a single exposure to a hormone can produce persistent alteration of the hormonal response characteristics or of prohormone processing [353, 372]. Maternal undernutrition during pregnancy in the rat results in the development of obesity, hyperinsulinaemia and hyperleptinaemia during adult life; this phenotype is potentiated when the offspring are fed a high-fat diet. Neonatal leptin treatment normalized the programmed phenotype, indicating that metabolic programming may be reversible during the period of developmental plasticity [373].

Maternal and fetal glucocorticoids have wide-ranging effects in the fetus, altering receptors, enzymes, ion channels and transporters in a variety of cells and tissues in the late gestation fetus and can induce programming of other endocrine systems. Throughout gestation, they modify *GLUT* gene expression in placenta and fetus, influence *IGF* and GR gene expression in various tissues, affect expression of several transcription factors and affect a wide variety of enzymes in placenta, liver, kidney, intestine and lung [374]. Maternal undernutrition, stress and placental dysfunction are associated with increased maternal and fetal glucocorticoid concentrations, which contribute to IUGR and programmed alterations in adult endocrine systems and metabolism [358, 374, 375].

Fetal Adaptations for Transition to Extrauterine Life

The transition from intrauterine to extrauterine life involves abrupt delivery from a protected environment with placental support into relatively hostile surroundings, and complex physiological processes are needed to ensure neonatal survival. All organ systems are involved to a degree, but the important immediate adaptations include clearance of fetal lung fluid, surfactant secretion and initiation of breathing in air, together with transition from a fetal to neonatal circulation. Other essential changes occur in endocrine function, substrate metabolism, and thermogenesis to protect against hypothermia,

hypoglycaemia and hypocalcaemia once the placental supply of energy and substrate is removed. In the immediate period post-partum, both the adrenal cortex and the autonomic nervous system are essential for adaptation to life *ex utero*. Longer-term transition requires maturation of the secretory control mechanisms for the PTH–calcitonin system and the endocrine pancreas.

Cortisol

Cortisol is the key regulatory hormone for terminal maturation of the fetus and neonatal adaptation at birth. In most mammals, a cortisol surge occurs due to a switch from maternally derived corticosteroids via the placenta to fetal adrenal production of cortisol under fetal hypothalamic control [111]. It has been proposed that the fetal cortisol surge is due to the progressive stimulation by oestrogens of placental HSD11B2 activity, resulting in an increase in placental conversion of cortisol to cortisone [111]. The subsequent decrease in maternal-to-fetal cortisol transfer results in stimulation of fetal CRH and pituitary ACTH secretion. Placental CRH may also potentiate fetal adrenal activation, and data suggest an increase in HSD11B1 expression and activity in placental and fetal membranes during late gestation, with a consequent increase in local cortisol production in preparation for parturition [376]. Cortisol concentrations progressively increase from 28 wpc to 34 wpc and rise further prior to the onset of parturition at term. Cortisol concentrations escalate during labour, peaking a few hours after delivery at term. The cortisol surge supports multiple physiological changes that facilitate normal neonatal adaptation (see Figure 3.13) [377, 378]:

- Augmentation of surfactant synthesis in lung tissue;
- Increasing lung fluid reabsorption;
- Increasing adrenomedullary PNMT activity, which in turn increases methylation of NA to adrenaline;
- Increasing hepatic iodothyronine outer-ring monodeiodinase activity and hence conversion of T_4 to T_3;
- Decreasing sensitivity of the ductus arteriosus to prostaglandins, facilitating ductus closure;

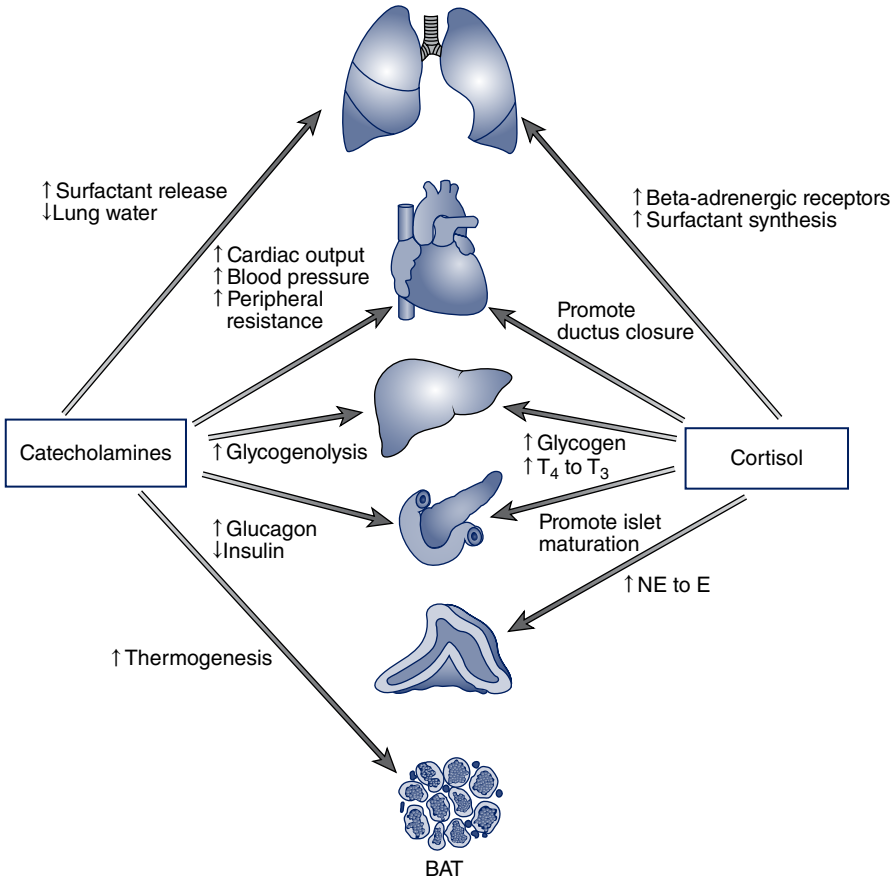

Figure 3.13 Actions of cortisol and catecholamines during fetal adaptation to the extrauterine environment. The prenatal cortisol surge acts to promote functional maturation of several organ systems. The neonatal catecholamine surge triggers or potentiates many the extrauterine cardiopulmonary and metabolic functional adaptations that are critical to extrauterine survival. BAT, brown adipose tissue; E, epinephrine; NE, norepinephrine; T_3, triiodothyronine; T_4, thyroxine.

- Inducing maturation of several enzymes and transport processes of the small intestine;
- Inducing maturation of hepatic enzymes.

Secondary effects of cortisol also promote postnatal adaptation. The increased T_3 concentrations stimulate β-adrenergic receptor binding, potentiate surfactant synthesis in lung tissue and increase the sensitivity of BAT to NE. The significance of fetal cortisol production is demonstrated by the effects of gene-targeted CRH or GR deficiency in mice; the progeny of homozygous CRH-deficient or GR-deficient animals die in the first 12 hours with lung dysplasia and surfactant deficiency [379, 380]. The known effects of the prenatal cortisol surge have led to the current recommendation for antenatal corticosteroid administration in pregnancies threatened by the risk of preterm delivery. Generally, preterm infants exposed antenatally to exogenous glucocorticoid therapy have lower morbidity and mortality overall compared with untreated infants.

Catecholamines

The human fetus at term can release catecholamines, including NA, adrenaline, and dopamine from adrenal medullary and other sympathetic tissues in response to fetal stress. Parturition evokes a catecholamine surge resulting in high concentrations in cord blood [193]. Plasma NA concentrations exceed adrenaline concentrations due to peripheral and adrenomedullary and paraaortic catecholamine release. Cord blood NA concentrations of 15 nmol/L (2500 pg/mL) and adrenaline concentrations of 2 nmol/L (370 pg/mL) are common after spontaneous delivery of term infants [193]. Concentrations of 25 nmol/L (4200 pg/mL) of NA and 35 nmol/L (640 pg/mL) of adrenaline have been measured in cord blood of premature infants. These changes evoke critical cardiovascular adaptations, such as increased blood pressure and cardiac inotropic effects, glucagon secretion, decreased insulin secretion, increased thermogenesis in BAT, pulmonary fluid mobilization and increased surfactant release [193, 376].

Thermogenesis

BAT is the major site of neonatal thermogenesis with the largest accumulations encasing the kidneys and adrenal glands and smaller amounts surrounding the blood vessels of the mediastinum and neck [381]. BAT mass peaks at term and gradually decreases during the first few weeks of life. Surgical removal of this tissue results in neonatal hypothermia. NE, acting via β-adrenergic receptors, stimulates BAT thermogenesis and optimal responsiveness is dependent on TH [382]. BAT is rich in mitochondria containing UCP1, a unique 32 kD protein (also known as thermogenin) that uncouples oxidation and phosphorylation of adenosine diphosphate, reduces ATP production and consequently enhances non-shivering thermogenesis [381]. UCP1 is T_3 dependent and BAT contains a 5′-monoiodothyronine deiodinase that deiodinates T_4 locally to T_3 [381]. Full maturation of catecholamine-stimulated cellular respiration in BAT occurs before delivery in the ovine fetus and requires TH [381]. Fetal thyroidectomy in this species leads to marked hypothermia, with low plasma free fatty acid concentrations and increased plasma adrenaline concentrations [383].

Rapid onset of non-shivering thermogenesis by BAT is essential for neonatal survival. Cord cutting, neonatal cooling, catecholamine stimulation and augmented conversion of T_4 to T_3 in BAT in the neonatal period are the essential features that mediate and condition newborn thermogenesis [382]. Fetal hypoxia and placental inhibitors, including prostaglandin E_2 and adenosine, appear to inhibit BAT thermogenesis *in utero* [382]. It was previously thought that involution of BAT occurred soon after birth but functional imaging using combined ^{18}F-fluorodeoxyglucose positron emission tomography (^{18}F-FDG-PET) and computed tomography (CT) modalities has identified active BAT in adults and demonstrated a strong positive correlation between basal metabolic rate and BAT activity [384–386].

Calcium Homeostasis

Neonatal adaptation includes adjusting rapidly from a high-calcium environment regulated by PTHrP and calcitonin to a low-calcium environment regulated by PTH and vitamin D. With removal of the placenta in term infants, plasma total calcium concentrations fall, reaching a nadir of approximately 2.3 mmol/L (9 mg/dL) [247], and ionized calcium concentrations fall to approximately 1.2 mmol/L (4.8 mg/dL) by 24 hours of life [387]. Plasma PTH concentrations are relatively low in the neonatal period and are minimally responsive to hypocalcaemia during the first 2–3 days of life. Calcitonin concentrations are high in cord blood (~2000 ng/L) and further increase remaining high for several days postpartum [247, 388]. The relatively blunted neonatal PTH response and high calcitonin concentrations result in a transient neonatal hypocalcaemia lasting 2–3 days [388, 389]. Subsequent inhibition of calcitonin secretion and stimulation of PTH secretion gradually result in a rise in serum calcium concentrations. The disappearance of PTHrP in the neonatal lamb coincides with the time of restoration of calcium concentrations to adult range [247]. The mechanism of transition from PTHrP to PTH secretion by the neonatal parathyroid glands however is unclear.

In the human neonate, calcium homeostasis is also affected by the low GFR that persists for several days after delivery [388, 389]. Furthermore, responsiveness to PTH is reduced in the first few days of life. These factors limit phosphate excretion and predispose the term neonate to hyperphosphataemia. Premature infants are found to have lower PTH and higher calcitonin concentrations and more immature kidney function. Neonatal hypocalcaemia in preterm infants may therefore be more marked and prolonged with a higher incidence of symptomatic hypocalcaemia. Additional predisposing factors to neonatal hypocalcaemia include birth asphyxia [389] and maternal hypercalcaemia related to hyperparathyroidism. In the latter case, infants have marked suppression of parathyroid function and a longer period of transient hypoparathyroidism in the neonatal period. PTH secretion and calcium homeostasis usually return to normal within 1–2 weeks in term infants and within 2–3 weeks in the preterm infant.

Glucose Homeostasis

In utero there is little endogenous fetal glucose production; however glucose and other substrates are stored as fat and glycogen in preparation for birth. During labour the secretion of stress hormones such as glucocorticoids and catecholamines causes a rise in fetal blood glucose concentrations. Clamping of the cord at delivery abruptly terminates the maternal supply of glucose. A healthy term infant experiences an immediate postnatal fall in blood glucose concentration during the first 2–4 hours from those close to maternal concentrations to around 2.5 mmol/L (45 g/dL) [228, 229]. Low blood glucose concentrations are usually transient, asymptomatic and part of the normal adaptation to extrauterine life. In term infants alternative fuels are produced and the transition to intermittent feeding and fasting with the introduction of milk feeds into the gut is accomplished in a seemingly straightforward manner. Counter-regulatory hormones rapidly become active with high catecholamine, glucagon, GH and glucocorticoid concentrations and a fall in insulin [228, 229]. Insulin concentrations are low at birth and normally fall further in response to hypoglycaemia. The early glucagon rise is brief but concentrations remain at about 100 ng/L for the first 12–24 hours and the glucagon/insulin ratio is high enough to stabilize blood glucose concentrations in the range of 2.8–4 mmol/L (50–70 mg/dL) during this period. The early glucagon and catecholamine surges deplete hepatic glycogen stores. Return of plasma glucose concentrations to normal after 12–18 hours requires increasing hepatic gluconeogenesis, which is stimulated by a high plasma glucagon/insulin ratio [229]. Glucagon secretion gradually increases during the early hours after birth, particularly due to milk protein feeding, which stimulates gut glucagon release and pancreatic glucagon secretion [228, 229]. Together, these mechanisms stabilize the blood glucose profile, although adult glucose concentrations are not reached until approximately 72 hours of life.

Neonatal hypoglycaemia can result due to failure of this sequence of physiological changes and is most commonly seen during the first few hours after birth. When an infant is born prematurely or following IUGR, the mechanisms of glucose homeostasis are compromised. Preterm infants are at risk of more severe and prolonged hypoglycaemia as they have reduced glycogen stores and impaired hepatic gluconeogenesis. Infants of diabetic mothers have more severe neonatal hypoglycaemia due to relative HI. In the healthy term infant, glucose homeostasis is achieved within 5–7 days of life, while in contrast 1–2 weeks may be required in premature infants.

Other Endocrine Adaptations

Post-partum neonatal concentrations of oestrogens, progesterone, hCG and PL decrease. The fall in oestrogen is presumed to remove the major stimulus to pituitary PRL release. PRL concentrations decrease over several weeks, and this relatively delayed fall may be due to lactotroph hyperplasia in the neonatal pituitary or to delayed maturation of hypothalamic dopamine secretion. The gradual fall of GH concentrations during the early weeks of life is due to delayed maturation of hypothalamic–pituitary feedback regulation of GH release [36]. In the neonatal primate, there are concomitant decreases in plasma GH concentrations and GH responsiveness to exogenous GHRH [390], although the mechanisms for this remain unclear; changes in secretion or in pituitary sensitivity to GHRH or somatostatin, or both, may be involved. IGF1 and IGF2 concentrations fall to values maintained in infancy within a few days, presumably because of the removal of PL and placental IGF production (see Figure 3.3).

In male infants a transient fall in testosterone concentrations is evident as the hCG stimulus subsides (see Figure 3.9). Subsequently, pituitary LH secretion rebounds and there is a surge of plasma testosterone that persists for several weeks [36, 180]. This surge is mediated by hypothalamic GnRH. In neonatal monkeys, blockade with GnRH agonist ablates neonatal activation of the hypothalamo–pituitary–gonadal axis, resulting in minimal increments of LH and testosterone [391]. Such a blockade also results later in subnormal increments in plasma LH and testosterone concentrations and poor testicular enlargement at puberty in these animals, suggesting that neonatal GnRH release with pituitary–testicular activation may be critical for normal sexual maturation of male primates [391]. In females, a

transient, secondary surge in FSH may transiently elevate oestrogen concentrations.

Delivery results in high plasma cortisol concentrations in the neonate despite relatively lower plasma ACTH concentrations (see Figure 3.5). The adrenal cortex atrophies and plasma DHEA-S and DHEA concentrations fall.

An increase in serum TSH concentrations seen during the first few minutes after birth is due to cooling of the neonate in the extrauterine environment [22, 122]. In term infants, the TSH surge peaks at 30 minutes at a concentration of about 70 mU/L (see Figure 3.7). This peak evokes increased secretion of T_4 and T_3 by the thyroid gland. In addition, increased conversion of T_4 to T_3 by liver and other tissues maintains the T_3 concentration in the extrauterine range of 1.6–3.4 nmol/L (105–220 ng/dL). During the first few weeks of life, readjustment of serum T_3 concentrations and maturation of feedback control of TSH by THs re-equilibrates neonatal TSH concentrations [22, 392]. Production of rT_3 by neonatal tissues subsides by 3–4 weeks of age, at which point serum rT_3 reaches adult concentrations.

Frontiers in Fetal and Neonatal Endocrinology

Fetal medicine as a specialty has seen tremendous advances in diagnostic and therapeutic procedures over the past few decades. An appreciation and growing understanding of the uniqueness of the endocrine milieu during pregnancy, coupled with explosions in the fields of fetal imaging, genomics and minimally invasive techniques, have reformed the management of some fetal and neonatal endocrine conditions. Furthermore, an understanding of developmental endocrinology and the natural history of many endocrine diseases is increasingly relevant in the management of preterm infants and those with IUGR, as well as elucidating the pathogenesis of many adult endocrine diseases.

Direct access to the intrauterine environment, and potential manipulation of this, heralds a new era in the provision of fetal therapy, with the caveat of limited evidence at present for the benefits and the risk of adverse effects [393]. Amniotic fluid fetal cell sampling, maternal plasma DNA analysis and intrauterine fetal blood sampling have resulted in breakthroughs in antenatal fetal diagnosis [394]. Non-invasive technologies for fetal evaluation, such as analysis of free fetal DNA from maternal blood and analysis of fetal products accessible at maternal sites, are realizing the promise of lower-risk robust diagnostics. Postnatally, assessment of antenatal environmental and/or drug exposures to the fetus can be retrospectively assessed by analysis of meconium, hair and other matrices [395]. Intrauterine diagnosis of fetal adrenal and thyroid disorders is becoming the standard of care [396, 397], and the prospect of *in utero* treatment is closely following, although this is controversial. Management of fetal hypothyroid goitre, for example, poses significant risks (including miscarriage) associated with both diagnostic amniocentesis/cordocentesis and intra-amniotic TH therapy [398]. These risks often preclude major studies of optimal prenatal therapy. Animal models are providing some evidence for novel fetal therapies, although the field is still at an experimental phase and careful consideration must be given to the ethical and safety concerns, as well as the practicalities of clinical translation to human trials. Intravenous nutritional supplementation of fetal sheep, for example, can prevent some forms of growth restriction and chronic fetal therapy through indwelling pumps is feasible [399].

These approaches, coupled with increasing availability of synthetic hormones and growth factor agonists and antagonists, could facilitate direct fetal endocrine therapy. The fetus in early gestation is a favourable recipient of cellular therapy and fetal cell transplantation, utilized successfully in the correction of congenital haematological diseases; this may be applicable as therapy for selected endocrine diseases [400]. Studies involving the endocrine pancreas, for example, have demonstrated long-term circulating human insulin following intrauterine stem cell transplantation in sheep, with potential translation as diabetic cellular therapy [401].

Finally, the role and applications of fetal gene therapy are beginning to emerge as a novel treatment strategy, offering the potential of cure in certain diseases, as well as the possibility of preventing irreversible organ damage that can occur antenatally or shortly after birth [402]. Intra-placental gene therapy, for example, takes advantage of an organ that will be discarded at birth. The results of preliminary studies in the rabbit have demonstrated that placental gene therapy may be an effective therapy for IUGR [403].

Medical treatment often advances through the resolution to experiment with novel techniques, technology, and practice beyond established boundaries. Fetal intervention is one of the most exciting evolving disciplines; however this field also raises complex ethical and medico-legal challenges, due to the existence of multiple grey zones, highlighting the need for clear, responsible evidence-based guidelines [404]. There has been advancement of the legal status of the fetus in the United Kingdom, Europe and the United States, in parallel with the growing specialty of fetal–maternal medicine [405]. Fetal intervention undertaken for research is governed and protected by strict ethical codes, but as the specialty becomes established, it will necessarily attract more interest from the law. Clinicians do not dictate legislation; however a solid framework of clinical principles can

help to shape future legislation [405]. The dissemination of information and findings internationally at scientific meetings for peer review, and involvement of relevant patient/public interest groups, is crucial. In this way evidence will direct consensus on practice, with active drive of treatments with the greatest potential into robustly conducted clinical trials, coupled with thoughtful, safe practice delivering excellence in care.

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4

Disorders of Sex Development

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KEY LEARNING POINTS

After having read this chapter, the reader should:

- Understand the basic principles of human gonadal differentiation, gonadal hormone production and action.
- Be familiar with the currently used classification of DSD conditions and terminology and be able to choose sensitive wording in their communication with affected individuals.
- Be aware of the recent changes in medical approach and the prevailing challenges and controversies in management.
- Have an understanding of basic mechanisms underlying gonadal germ cell tumour development in DSD patients and identify major risk groups.
- Be able to develop a basic diagnostic and management plan for a newborn who has atypical genital characteristics, in accordance with the facilities and limitations of the institute he/she is working in and in communication with the regional specialized DSD team.

Disorders of sex development (DSD) may present in the newborn period with atypical genitalia but may also be diagnosed later in childhood or adolescence; they may require lifelong multidisciplinary attention. The terminology, classification and management of DSD have been controversial over the last two decades and have been thoroughly revised, although not yet finally decided. Major progress has been made in understanding the genetic causes and mechanisms of DSD, yielding information that is being gradually translated into individualized care. Patient advocacy groups and activists, as well as societal and political perceptions on the topic, have placed ethical issues equally high on the agenda.

For a more detailed and practical understanding of DSD, the authors refer the reader to the DSD chapter of the ESPE e-learning module http://www.espe-elearning.org/.

Normal Sex Development

The embryology of the reproductive system and the genetic and hormonal control of its constituent parts are described in detail in recent texts and reviews [1–4] and the clinician charged with the evaluation and management of DSD needs a detailed knowledge of reproductive tract development, which underpins differential diagnosis, focused investigation and appropriate management. An animated module, describing normal and atypical sex development with focus on gonadal (mal)development, is available on http://www.erasmusmc.nl/pathologie/research/lepo/4530687/.

Differentiation of the bipotential gonad into a testis or ovary requires time- and dose-critical activation of promoting and/or suppressing genetic cascades triggered by the presence or absence of the sex-determining gene on Y (*SRY*). Subsequent development of the male internal reproductive tract and external genitalia depends on the timely and sufficient production and action of testicular hormones, in the absence of which a female internal reproductive tract and external genitalia develop (Figures 4.1–4.3).

In the presence of an XY karyotype, causes of DSD include defects in testis determination or in the production or action of testicular hormones, mainly androgens [4, 5]. Common elements in the early pathway (e.g. *WT1* and *NR5A1*) explain the importance of checking renal and adrenal disorders when evaluating a DSD. In the case of an XX karyotype, a primary defect in ovarian determination may be the cause but masculinization of the XX fetus by fetal adrenal androgens (e.g. congenital adrenal hyperplasia [CAH]) or androgens from a maternal source

† Deceased

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Companion website: www.wiley.com/go/dattani/brookcpe7

(e.g. ovarian tumour) is far more frequent. It becomes increasingly clear that proper sex determination is secured by continuous repression of the opposite pathway. Thus, loss of activation of one pathway inevitably implies partial activation of the opposite pathway due to loss of antagonism of the other. The concept that some genetic defects (e.g. *NR5A1*) may lead to both XY defective testis development and XX primary ovarian insufficiency (POI) is relatively new and it is expected that similar mechanisms will be described for other genes in the near future.

Sex chromosome abnormalities, such as X/XY mosaicism or sex chromosome aneuploidy, may be associated with atypical sexual and/or genital development.

Terminology

The word '*intersex*', perceived as uninformative by some and stigmatizing by others, was replaced by the acronym 'DSD' (for '*disorders of sex development*') in 2005 [6]. It is defined as a congenital condition in which the development of chromosomal, gonadal or anatomical sex is atypical, and, as a medical term, it is generally accepted nowadays and is used as such in this chapter. However, many advocacy groups point out that medical and societal concepts of (binary) sex and gender may be perceived as inappropriate by individuals with atypical genital characteristics. In line with a tendency to de-medicalize these conditions, terms such as '*differences*' or '*variations*' of sex development have been proposed. Individuals living with a DSD tend to use the specific name of their condition rather than the generic term DSD.

Early knowledge of the sex chromosomes from the analysis of Y and X centromeric probes by fluorescent *in situ* hybridization (FISH) techniques renders this the starting point for the evaluation of a DSD, which will subsequently be categorized as 46,XY, 46,XX or sex chromosomal DSD. Confusing terms such as pseudohermaphroditism and true hermaphroditism have been abandoned. In many cases, a more precise diagnosis can be obtained using new diagnostic technologies. The further diagnostic workup is aimed at differentiating conditions caused by defective or incomplete gonadal differentiation from those resulting from decreased or excess hormone production or action. As new forms and causes emerge, classification of DSD is subject to regular changes and updates. An example is presented in Table 4.1.

Postnatal psychosexual development and sociocultural influences on gender have spawned their own terminology. It is important to be familiar with these concepts when considering the evaluation and management of DSD and when counselling affected individuals and their parents. Transgenderism and DSD are distinct conditions with a divergent aetiology; most people

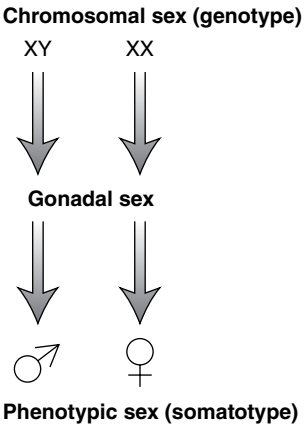

Figure 4.1 Schematic representation of the fundamental components of sex development.

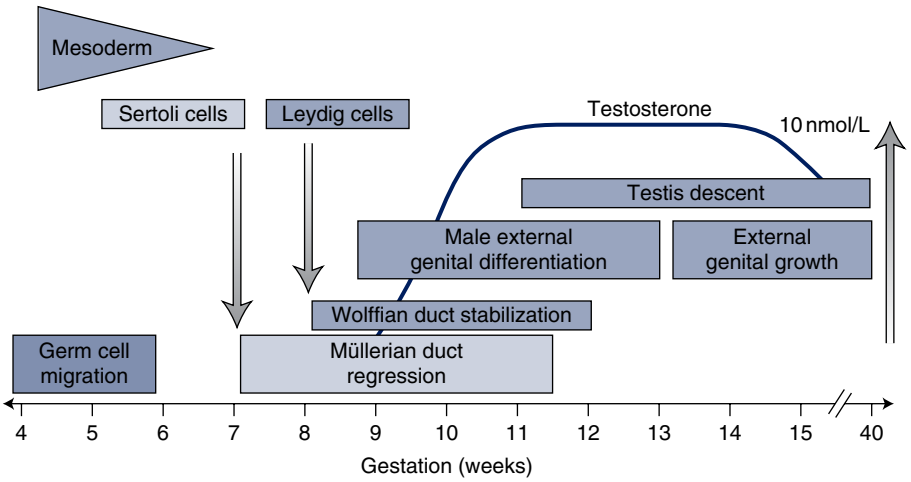

Figure 4.2 Events temporally related to sex differentiation in the male fetus. Mesoderm refers to the tissue source for Sertoli and Leydig cell formation. The continuous line depicts the rise in fetal serum testosterone, the peak concentration being around 10 nmol/L.

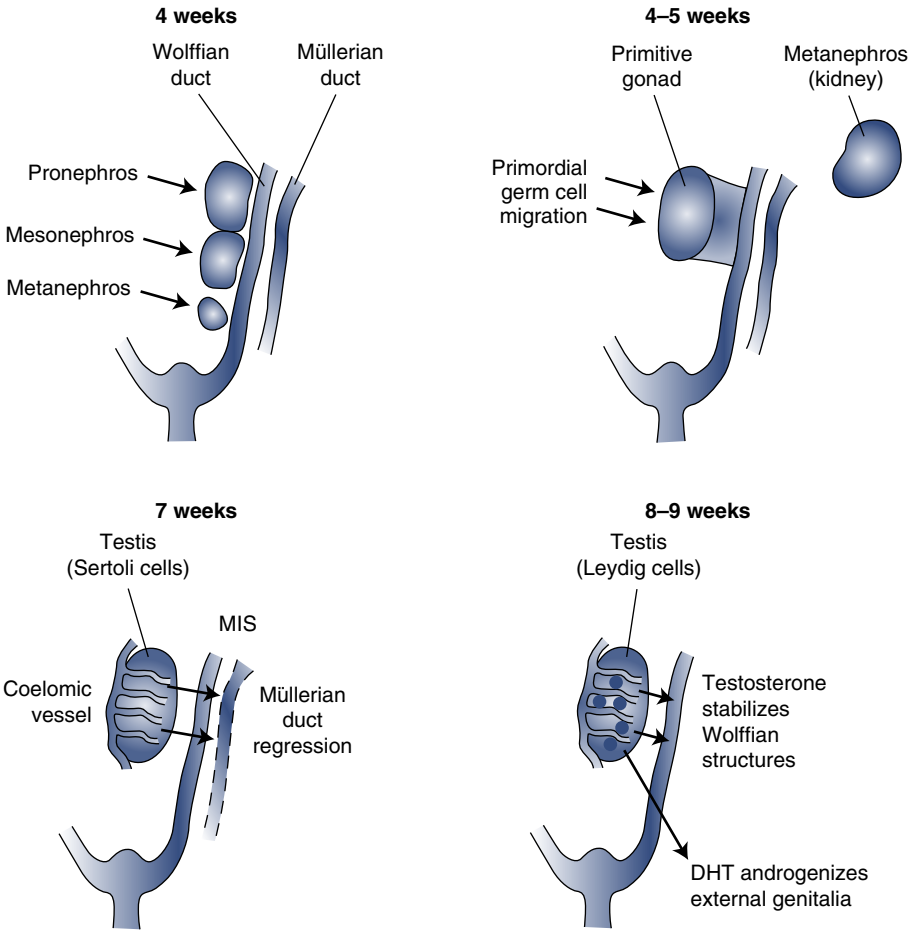

Figure 4.3 Schematic representation of the principal morphologic and functional events during early gonad/testis development in humans. DHT, dihydrotestosterone. *Source:* From Hughes and Achermann [1], with permission.

with unexpected genital characteristics do not experience gender dysphoria [7]. Controversy exists regarding the use of the terms 'sex' and 'gender', the first putting more emphasis on the biological aspects and the latter emphasizing the psychosocial components of sex/gender:

- *Gender (sex) assignment* is the decision of gender (sex) of rearing usually taken instantaneously at birth and thus with uncertainty about a person's future gender identity.
- *Gender identity* is the sense of self as being male, female or in between.
- *Gender role* denotes aspects of social behaviour in which males and females differ or are expected to behave differently.
- *Sexual orientation* refers to a person's natural preference in sexual partners.
- *Gender dysphoria* is the discomfort or distress caused by a discrepancy between a person's gender identity and their gender (sex) of rearing.

Causes of DSD

A practical approach to understanding DSD and its causes and consequences is to consider separately:

a) Conditions caused by impaired formation of the bipotential gonad or its subsequent differentiation into either testis or ovary, including conditions with sex chromosome mosaicism.
b) Conditions in XY individuals characterized by testis development but in whom there is insufficient testosterone production or action.
c) Conditions in XX individuals associated with normal ovarian development and function but in whom virilization occurs due to excess androgen production of extragonadal, mostly adrenal, origin (CAH) (see Table 4.1).

Most DSDs can be classified within these groups, although some clinical entities remain, such as Mayer–Rokitansky syndrome and isolated hypospadias.

Table 4.1 Biological classification of DSD.

46,XY DSD	46,XX DSD	Sex chromosomal DSD
Disorders of gonadal development	**Disorders of gonadal development**	**45,X**
Ovotesticular DSD	(Ovo)testicular DSD	Turner syndrome and variants
Complete or partial gonadal dysgenesis, monogenic forms (SRY, NR5A1, WT1, etc.)	Monogenic forms of primary ovarian insufficiency (mutations in genes involved in gonadal (ovarian) development, e.g. NR5A1, WT1, etc.)	
Testis regression		
Syndromic forms	Syndromic forms	
Disorders of androgen synthesis	**Disorders of androgen excess**	**47,XXY**
Associated solely with androgen biosynthesis defects (mutations/deficiencies in HSD17B3, SRD5A2, etc.)	Aromatase deficiency	Klinefelter syndrome and variants
Associated with congenital adrenal hyperplasia and early androgen biosynthesis defects (mutations/deficiencies in STAR, CYP11A1, HSD3B2, POR, CYP17A1, etc.)	Congenital adrenal hyperplasia (mostly mutations/deficiencies in CYP21A2)	
	Luteoma	
Associated with placental insufficiency or endocrine disruption	Iatrogenic	
Syndromic forms (e.g. Smith–Lemli–Opitz)		
Disorders of androgen action		**45,X/46,XY and 46,XX/46,XY**
Complete and partial androgen insensitivity		Mixed gonadal dysgenesis Chimerism
Unclassified disorders	**Unclassified disorders**	**Other complex chromosomal rearrangements**
Hypospadias of unknown origin	Mayer–Rokitansky type I and II syndrome	
Epispadias	Complex syndromic disorders	
Persistent Müllerian duct syndrome		
Complex syndromic disorders		

Abbreviations: DSD, disorders of sex development; SRY, sex-determining region Y; NR5A1, nuclear receptor subfamily 5, group A, member 1; WT1, Wilms' tumour 1; HSD17B3, hydroxysteroid (17-beta) dehydrogenase 3; SRD5A2, steroid-5-alpha-reductase, alpha polypeptide 2; CYP11A1, cytochrome P450, family 11, subfamily A, polypeptide 1; STAR, steroidogenic acute regulatory protein, hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 2; POR, P450 (cytochrome) oxidoreductase; CYP17A1, cytochrome P450, family 17, subfamily A, polypeptide 1; CYP21A2, cytochrome P450, family 21, subfamily A, polypeptide 2.

Turner and Klinefelter syndromes are included in DSD classification because of their sex chromosome constitution. These patients are often infertile due to gonadal dysgenesis but they also display condition-specific comorbidities, which makes them a separate group. They benefit from multidisciplinary care as do typical DSD patients but they are often not perceived as having a DSD and these conditions are covered in Chapter 7.

Impaired Gonadal Development

XY Individuals

Complete and Partial Gonadal (Testicular) Dysgenesis

Failure of the bipotential gonad to develop or to differentiate into a functional testis in an XY individual gives rise to gonadal dysgenesis. Normal development and function of Sertoli and Leydig cells are essential for hormone-mediated sex differentiation of male internal and external genitalia and these processes are disturbed to a variable extent, which determines the phenotype. Gonadal histology is equally variable and may reflect loss of antagonism of the opposite (female) pathway together with incomplete testis differentiation (Figure 4.4). The spectrum of gonadal differentiation may be highly variable, even within the same gonad, and should be regarded as a continuum of many (all) possible outcomes, possibly reflecting micro-environmental influences. Sometimes the gonadal precursors undergo regression and no gonadal tissue can be found at laparoscopy.

Streak gonads comprise mainly fibrous tissue with no germ cells, Sertoli cells, interstitial steroid-secreting cells, tubules or follicles. When both gonads are streaks or absent, no fetal hormones are produced and the phenotype at birth is female. Consequently, XY complete

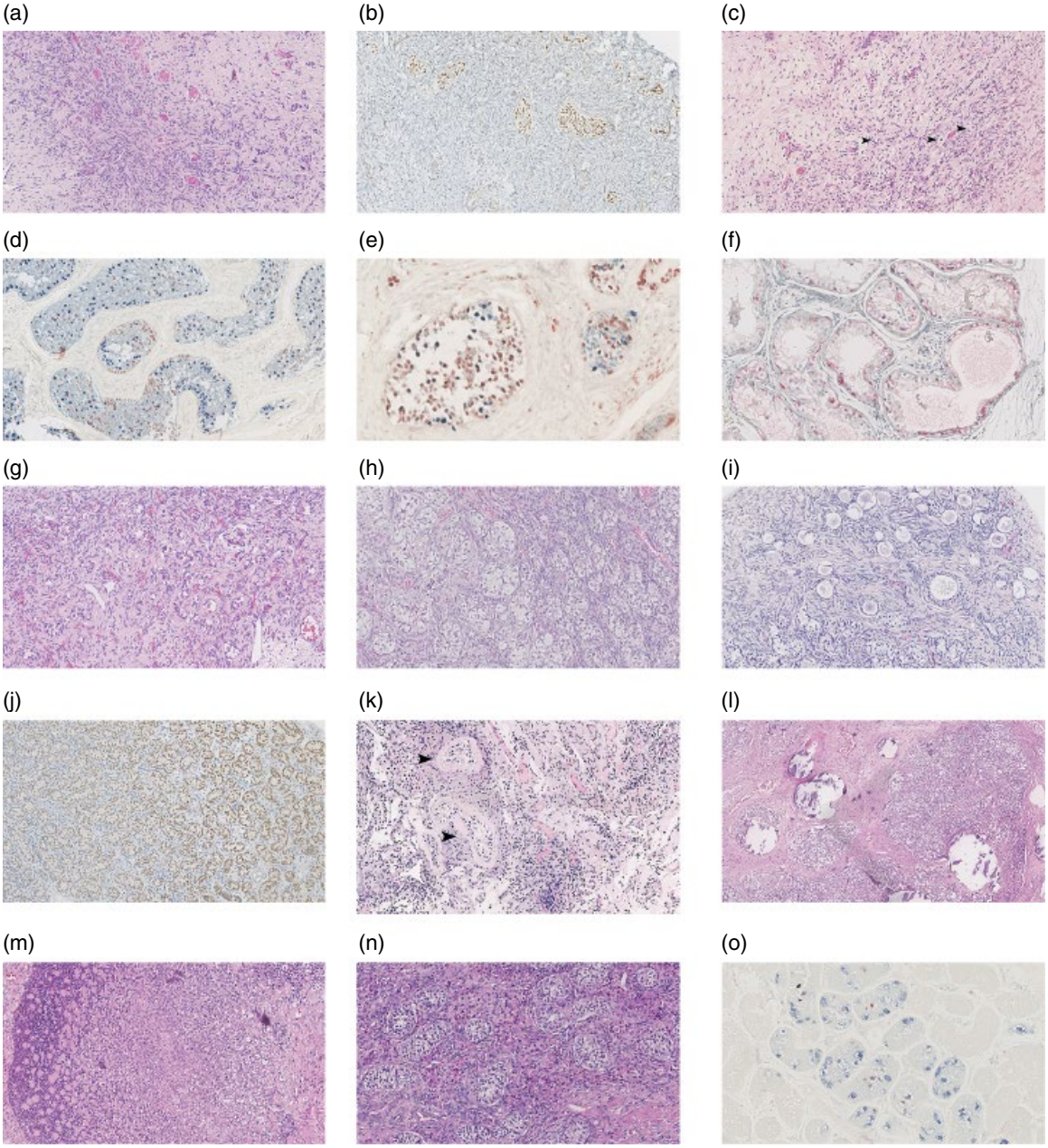

Figure 4.4 Examples of gonadal differentiation patterns, illustrating the variability of possible gonadal outcomes and co-presence of characteristics of both the male and female pathways in many dysgenetic gonads, as opposed to gonads from individuals with hormonal defects. (a) Streak gonad in an individual with 45,X/46,XY mosaicism. HE staining, 200×. (b) Streak gonad in another individual with 45,X/46,XY. Some cells have differentiated as Sertoli cells (SOX9 positive, brown staining) and form tubular aggregates. SOX9 staining, 200×. (c) Undifferentiated gonadal tissue in a 46,XY individual with *WWOX* mutation. Germ cells (arrowheads) form aggregates with pre-Sertoli/granulosa cells in a background of fibrous stroma. Testis tubules or ovarian follicles cannot be readily recognized. HE, 200×. (d) Partial gonadal dysgenesis in a 46,XY individual of unknown aetiology. Testis tubules contain both cells with Sertoli (blue) and granulosa (red) cell differentiation. SOX9–FOXI2 double staining, 200×. (e) Gonadoblastoma in the same individual. Supporting cells in a gonadoblastoma context are mostly (pre-)granulosa cells. SOX9–FOXI2 double staining, 400×. (f) Carcinoma *in situ* (CIS) in the same individual. Premalignant germ cells (TSPY positive, red staining) are stuck to the thickened basal membrane; the supportive Sertoli cells are shifted away from the basal membrane towards the internal side of the germ cell layer. Note that the supporting cells in a CIS context are Sertoli cells as opposed to gonadoblastoma where the supportive cell line consists of (mainly) granulosa cells. (g) Area of undifferentiated gonadal tissue in an individual with 45,X/46,XY. Many germ cells can be recognized, sometimes isolated in fibrous stroma, and sometimes arranged in clusters with pre-Sertoli/pre-granulosa cells. HE staining, 200×. (h) Gradual transition from testis (left) to a more undifferentiated pattern (right) in an individual with 45,X/46,XY. HE, 200×. (i) Ovotesticular DSD in a 46,XX SRY-negative individual. Ovarian differentiation in the upper part, testis in the lower part. HE, 200×. (j) Testicular DSD in a 46,XX SRY-negative individual. In spite of the absence of SRY, there is clear SOX9 expression (brown staining) in small (infantile) Sertoli cell-only tubules. SOX9 staining, 200×. (k) Testis atrophy with loss of testicular architecture in an older (40 years) individual with 46,XX testicular DSD. Scarce hyalinized atrophic Sertoli cell-only tubules (arrowheads) in a background of fibrous stroma. HE, 200×. (l) Large gonadoblastoma in an individual with 45,X/46,XY. HE, 100×. (m) Same gonad, dysgerminoma component. HE, 200×. (n) Typical testis differentiation but with marked Leydig cell hyperplasia in a 46,XY adolescent with CAIS. The intertubular space is packed with Leydig cells. HE, 200×. (o) Testis of a 3-year-old CAIS individual showing maturation delay of germ cells (gonocytes) in luminal position, inappropriately expressing OCT3/4 (red staining) well beyond birth, next to more mature spermatogonia at the basal membrane, expressing TSPY (blue cells). OCT3/4 – TSPY double staining, 200×. (*See insert for colour representation of the figure.*)

gonadal dysgenesis (formerly referred to as 'Swyer syndrome') leads to the development of typical female internal and external genital structures and a female gender identity in almost all patients. The diagnosis is often made at adolescence following evaluation of absent pubertal (including the breast) development.

Partial gonadal dysgenesis gives rise to ambiguity of the external genitalia because of the preservation of some Leydig cell function. There may be Müllerian duct remnants, reflecting inadequate Sertoli cell production of anti-Müllerian hormone (AMH). Histology shows a thin and loosely organized tunica albuginea, underdeveloped seminiferous tubules with branching tubules, wide intertubular spaces, abundant infantile Sertoli cells and scanty or absent germ cells. In some cases, the gonadal architecture is so underdeveloped that it is reminiscent of primitive sex cords rather than immature testis tubules, often also displaying isolated primordial germ cells without any apparent organization in sex/ovigerous cords. This is referred to as 'undifferentiated gonadal tissue' or sometimes erroneously as 'ovarian-type stroma' and should not be mistaken for ovarian differentiation, which implies the presence of meiotic germ cells organized in follicles.

Although these appearances share features with an early developing gonad and may thus represent a simple failure in gonad maturation, the supportive cell line often contains cells with Sertoli (SOX9 positive) as well as granulosa (FOXL2 positive) cell differentiation, suggesting incomplete suppression of the female pathway together with failure of testicular development [8] (Figure 4.4). There is a high risk of gonadal tumours such as the precursor lesion gonadoblastoma and its invasive counterparts seminoma, non-seminoma and dysgerminoma in these individuals, especially in cases who have undifferentiated gonadal tissue.

Many genes have been involved in early gonadal stabilization and developmental pathways and may cause 46,XY DSD due to complete or partial gonadal dysgenesis when mutated (reviewed in [2, 4]). 10–15% of patients have a mutation of the *SRY* gene, usually in the HMG-box DNA-binding domain of the SRY protein [9, 10]. There are familial cases of XY complete gonadal dysgenesis in whom the genetic cause is unknown and the pattern of inheritance can be X-linked or autosomal recessive [11].

Steroidogenic factor 1 (*SF1*, alternatively named *NR5A1*) is a nuclear receptor that regulates transcription of several genes involved in both gonadal and adrenal development and in steroidogenesis. The expected phenotype of XY complete or partial gonadal dysgenesis in combination with adrenal insufficiency has been observed very rarely in patients with (mostly) homozygous or compound heterozygous *SF1* mutations. Heterozygous mutations and deletions in *SF1* are a frequent cause of partial gonadal dysgenesis with normal adrenal function. The mechanism is likely to be haploinsufficiency, the gonad being more sensitive than the developing adrenal to variations in gene dosage effects. The phenotype and hormonal workup in individuals with heterozygous *SF1* mutations may show large overlap with partial androgen insensitivity syndrome (PAIS) in the neonatal period but individuals with *SF1* mutations and retained gonads may experience important virilisation, including phallic growth, at puberty which PAIS patients generally do not. Heterozygous *SF1* mutations have been found sporadically in males with bilateral anorchia or isolated micropenis. Although often inherited from the mother in a sex-limited manner, it has been shown recently that *SF1* mutations in XX individuals may lead to POI [12–14]. An association with asplenism has been described occasionally [15]. A website summarizing available scientific data on *SF1* is currently under construction: http://www.steroidogenicfactor-1.info.

Two disorders to be considered in patients with partial or complete gonadal dysgenesis in association with renal abnormalities are the Denys–Drash and Frasier syndromes, both caused by mutations in *WT1*, a gene essential for gonadogenesis and nephrogenesis. In the Denys–Drash syndrome, there are usually genital anomalies at birth in XY cases, a characteristic nephropathy caused by diffuse mesangial sclerosis and a predisposition to Wilms' tumour at a median age of onset by 12 months. There is a relative 'hotspot' within exon 9 of the gene where most of the heterozygous mutations occur. In XY individuals with Frasier syndrome, there may be severe partial or even complete gonadal dysgenesis, a nephropathy characterized by focal segmental glomerulosclerosis and a predisposition to gonadal tumours rather than Wilms' tumour. End-stage renal failure usually occurs in the second decade of life.

The characteristic *WT1* abnormality in Frasier syndrome is caused by a donor splice site mutation in intron 9, which leads to an alteration in the normal ratio of WT1 protein isoforms. A *WT1* mutation can occur rarely in isolated hypospadias without evidence of nephropathy or a Wilms' tumour. The rarity of this occurrence does not merit screening for *WT1* mutations in all infants with hypospadias, although there may be a case for prospective screening for nephropathy (proteinuria) and Wilms' tumour (renal ultrasound) in XY infants with ambiguous genitalia or isolated proximal hypospadias.

The WAGR syndrome (Wilms' tumour, aniridia, genital anomalies, mental retardation) is a contiguous gene deletion syndrome involving a chromosome 11p locus that includes the *WT1* and *PAX6* genes [16–18].

Other examples of mutated genes causing syndromic forms of DSD are *SOX9*, *ATRX*, *DMRT1* and *GATA4*. Campomelic dysplasia is a multisystem disorder, caused by heterozygous mutations in *SOX9*, an *SRY*-related

transcription factor involved in both chondrogenesis and early testis determination. Not all affected patients have gonadal dysgenesis. This is related to the function of the SOX9 protein binding to DNA either as a dimer or as a monomer with dimerization being mandatory for chondrogenesis but not gonadal development.

ATR-X syndrome comprises alpha thalassemia, mental retardation and gonadal dysgenesis syndrome caused by mutations in the X-encoded *ATRX* gene.

Deletions of 9p encompassing the *DMRT1*, *DMRT2* and *DMRT3* genes may lead to a complex phenotype including mental retardation and variable degrees of gonadal dysgenesis. The respective roles of these genes relevant to the various phenotypic features of the so-called 9p monosomy syndrome remain to be elucidated but *Dmrt1* orthologues are involved in sex determination in other species (*Drosophila*, *C. elegans*) and, although not required for testis determination in mice, sustained Dmrt1 expression in the adult mouse testis is required to maintain its architecture.

Heterozygous mutations in *GATA4* and its cofactor *FOG2* have been described in individuals with partial gonadal dysgenesis sometimes associated with congenital heart defects [19].

Testis Regression (Anorchia)

Embryonic testis regression is considered in 46,XY individuals with a typical male phenotype or a short penis in whom no testis can be found on one or both sides at birth or later in life. The presence of a penile urethra suggests that testes were present in the first trimester [20]. Most cases are thought to result from a vascular accident with testicular torsion later during pregnancy [21]. Familial cases or a family history of infertility and cryptorchidism has been described but a causative gene mutation has been documented in only one case [22].

The diagnosis is confirmed by increased FSH in infancy or from puberty onwards with low values for AMH and/or inhibin B and for testosterone after human chorionic gonadotropin (hCG) stimulation [23]. Laparoscopic exploration may reveal the presence of a small fibrous nodule where a testis would be expected but such a diagnostic intervention is not usually necessary. Whether such fibrous nodules should be excised in view of an increased germ cell cancer risk is controversial. Viable germ cells have been demonstrated, especially in young children, but their survival is questionable. A practical approach is to remove possible testicular remnants in adolescence when testicular prostheses are inserted [24]. A short course of testosterone during infancy or childhood may result in some growth of a microphallus but whether this will lead to an increased adult penile length is not known [25].

XX individuals

Testicular and ovotesticular DSD

Eighty-five percent of individuals with XX testicular DSD have typical male genitalia and present as infertile adults ('XX males'), sometimes with gynaecomastia. Wolffian ducts develop normally and Müllerian structures are absent. Most of them harbour an Xp:Yp translocation containing *SRY*. Preferential inactivation of the Y-bearing X chromosome and cryptic mosaicism with SRY expression confined to the testes has been implicated in incomplete virilization.

In XX testicular DSD, a gonadal biopsy most often reveals small seminiferous tubules with Sertoli cell-only appearance and Leydig cell hyperplasia in pubertal patients. Sertoli cells invariably stain positive for SOX9, even in *SRY*-negative cases, indicating activation of the male testis determining pathway through other mechanisms (Figure 4.4j). In older individuals, seminiferous tubules become increasingly sclerotic and the normal testicular architecture is gradually replaced by general fibrosis (Figure 4.4k).

The term ovotesticular DSD should be reserved for individuals who have well-differentiated and functional testicular as well as ovarian tissue (i.e. Leydig cells and seminiferous tubules with or without germ cells and ovarian follicles composed of granulosa cells, theca cells and ova) either combined in an ovotestis (Figure 4.4i) or as asymmetrically developed gonads [8]. The testicular and ovarian parts both produce sex steroids at puberty leading to phallic growth and breast development. Large pedigrees have been described in which some individuals have ovotesticular DSD, some have testicular DSD, and others are asymptomatic carriers so it is believed that the condition is characterized by variable penetrance [26].

Ectopic SRY expression is not usually involved in conditions characterized by XX (ovo)testicular DSD and ambiguous genitalia. A few other genetic causes have been identified, such as gain-of-function changes of male sex-determining genes or its regulatory regions, e.g. *SOX9*, or loss-of-function mutations of female sex-determining genes, e.g. *RSPO1*, which has been implicated in a rare syndrome characterized by XX testicular DSD, palmoplantar hyperkeratosis and susceptibility to develop squamous cell carcinoma. Genomic rearrangements (duplications as well as a microdeletion) disrupting the regulatory region of *SRY*-related genes such as *SOX3* and *SOX10* have been described. It has been hypothesized that ectopic gonadal expression of these genes at a critical stage of development in these cases has triggered SOX9 transcription and subsequent initiation of the male pathway [19]. *NR5A1*, apart from its crucial role in testis development, has also been

implicated in activation of early ovarian-determining genes such as *WNT4*/β-catenin. Several families have been described in which a specific mutation in *NR5A1* c.274C > T (R92W) has led to ovotesticular or testicular DSD in some family members while others are asymptomatic carriers. Although the exact mechanism remains to be elucidated, it has been hypothesized that R92W specifically interferes with this NR5A1-mediated activation of ovarian development, resulting in loss of suppression of the testicular pathway in affected individuals [27, 28].

Ovarian and testicular differentiation, sometimes separated by a transitional zone, is the histological hallmark of ovotestes. FOXL2 and SOX9 immunopositive staining confirms granulosa and Sertoli cell differentiation, respectively. Germ cells are rarely present in the testicular part and tend to disappear before puberty; spermatogenesis is never observed, and testis tubules undergo hyalinization and sclerosis in adults. In contrast, follicles are usually abundant in the ovarian part in younger patients and may undergo ovulatory changes after puberty.

Ovarian Dysgenesis and Primary Ovarian Insufficiency

Although less well understood than testicular development, the differentiation of the bipotential gonad into a functional ovary has been associated with a number of genes, which, when mutated, give rise to ovarian dysgenesis (reviewed in [29, 30]). POI, characterized by accelerated follicular atresia following follicular damage and resulting in the formation of a fibrous streak, is not commonly considered a DSD and an autoimmune or iatrogenic (e.g. after chemotherapy) origin has been identified in many cases. However, common mechanisms of gonadal maldevelopment underlying some forms of POI and 46,XY DSD are suggested by the finding that specific mutations in *NR5A1* causing 46,XY gonadal dysgenesis segregated with the occurrence of POI in female family members [13].

NR5A1 and *WT1* mutations have been demonstrated in some women with POI, suggesting that these but probably also other early sex-determining genes may underlie both testicular dysgenesis and POI [31, 32]. An increasing number of rare monogenic forms of POI have been identified, caused either by disruption of genes regulating folliculogenesis (e.g. *BMP15*, *NOBOX*, *FOXL2*, *NUP107*, *NANOS3*, *FIGLA*) or meiosis (e.g. *HFM1*, *MSH4*, *DMC1*, *TUBB8*), but numerical anomalies of the sex chromosomes and *FMR1* premutations resulting from expansion of the normal number of CGG repeats remain the most frequent forms. Overall, an estimated 10–20% of ovarian dysgenesis is thought to result from mutations in ovarian-promoting genes [30–32].

Numerical Abnormalities of the Sex Chromosomes

45,X/46,XY (and variants)

The condition associated with 45,X/46,XY mosaicism is often called 'mixed gonadal dysgenesis' because affected individuals may have a streak gonad on one side and a testis on the other. Gonadal histology in 45,X/46,XY individuals is far more complex and variable and can be appreciated only by detailed pathological description of the gonads as shown in Figure 4.4. The term should be abandoned.

Sex chromosome mosaicism (45,X/46,XY and variants) occurs with an estimated incidence of around 1.5/10,000 and may be due to loss of the Y chromosome through anaphase lag or to interchromosomal rearrangements with final loss of a structurally abnormal Y chromosome. Paternal transmission of an abnormal Y chromosome with subsequent loss of Y has been described but seems to be a rare cause of 45,X/46,XY [33].

Ninety-five percent of individuals with 45,X/46,XY are believed to present as normal males [33] and there is little information about the outcome of these cases in relation to growth, puberty, fertility and risk of gonadal tumours. The remainder are characterized by great phenotypic variability, ranging from newborns with isolated hypospadias or ambiguous genitalia to females with Turner syndrome. No clear correlation exists between the proportion of the different cell lines in peripheral blood, fibroblasts or even gonads and the observed phenotype [34]. Characteristics typically associated with Turner syndrome, such as short stature, short fourth metacarpal and metatarsal, horseshoe kidney, wide-spaced nipples and shield thorax, are often present. Of note, structural cardiac anomalies and cardiovascular pathology are present in 45,X/46,XY males to the same extent as in females with Turner syndrome and require similar follow-up [35]. Mild mental retardation, autism and facial dysmorphism may also be part of the phenotypic spectrum.

Gonadal histology is highly variable but correlates to a certain extent with the child's phenotype: 45,X/46,XY girls with Turner syndrome typically have bilateral streak or sometimes absent gonads; genital ambiguity is often associated with the presence of dysgenetic testes or undifferentiated gonads, whereas at least one scrotal testis is usually present in phenotypic males. Asymmetrical mesonephric and paramesonephric duct development in agreement with the ipsilateral gonad is common and this finding in a 46,XY individual should always prompt gonadal karyotyping to exclude gonadal sex chromosome mosaicism. Ovarian histology (germ cells enclosed in primordial and maturing follicles) is extremely rare in 45,X/46,XY [36, 37].

46,XX/46,XY Chimerism (and Variants)

46,XX/46,XY chimerism is defined as the existence of two or more cell lines of different genetic origins in one individual and arises at or immediately after fertilization. 46,XX/46,XY chimerism may lead to ovotesticular DSD.

Disturbances of Testicular Hormone Production or Action

Impaired Biosynthesis

The pathway for production of androgens by the fetal testis is shown in Figure 4.5. Fetal serum testosterone concentrations rise to the normal adult male range towards the end of the first trimester, leading to Wolffian duct stabilization and subsequent differentiation into the epididymis, seminal vesicles and seminal duct, followed later by growth of the external genitalia and prostate (Figure 4.2). The timing and magnitude of the rise in androgen and AMH concentrations are critical determinants of male sex differentiation. Abnormalities in biosynthetic steps can result in inadequate androgen production and an undervirilized 46,XY infant. Some of the abnormalities also affect adrenal steroidogenesis.

Fetal Leydig cell androgen synthesis is initially under the control of placental hCG but depends on luteinizing hormone (LH) stimulation from the fetal pituitary from the second trimester. Both ligands bind to a common receptor. Inactivating mutations in the *LHCGR* gene cause a spectrum of undervirilization in XY individuals ranging from a typical female phenotype to severe

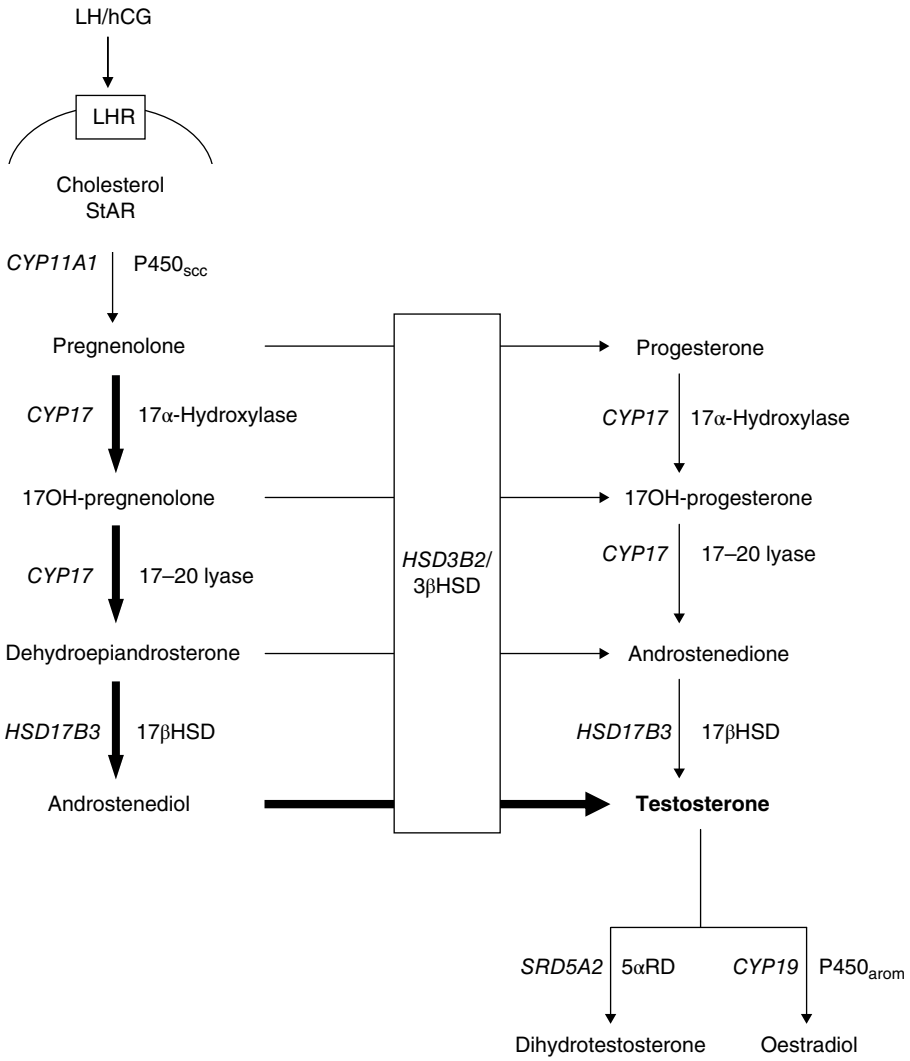

Figure 4.5 Pathways of testosterone synthesis in the human testis. The dominant pathway is indicated by the bold arrows. The enzymes encoded by their respective genes (italicized) are shown. LHR, LH receptor; P450scc, cytochrome P450 side-chain cleavage; 17βHSD, 17β-hydroxysteroid dehydrogenase; 3βHSD, 3β-hydroxysteroid dehydrogenase.

hypospadias with ambiguous genitalia, undescended testes or isolated micropenis. The expected endocrine profile is low testosterone and elevated LH concentrations with no testosterone response to hCG stimulation.

Testicular histology shows Sertoli cells with no interstitial Leydig cells. These features are difficult to confirm in the prepubertal child. It is intriguing that Wolffian ducts are stabilized despite the apparent lack of normal fetal testosterone production. Müllerian derivatives are absent due to normal testicular AMH production [38].

The early steps of cholesterol-mediated steroidogenesis are common to both the adrenals and gonads. Thus 46,XY infants with lipoid CAH resulting from StAR deficiency (due to a mutation in the *STAR* gene) or P450 side-chain cleavage deficiency (caused by *CYP11A1* gene mutation) present as phenotypic females. XX and XY individuals with combined 17α-hydroxylase/17,20-lyase deficiency typically present as females with lack of puberty, low renin hypertension and hypokalaemic alkalosis. Variable degrees of undervirilization are seen in 46,XY individuals with 3β-hydroxysteroid dehydrogenase deficiency (due to *HSD3B2* gene mutation), isolated 17,20-lyase deficiency (*CYP17* gene mutation), cytochrome B5 deficiency and P450 oxidoreductase (POR) deficiency. POR is a key electron donor enzyme providing electrons to all P450 (CYP) enzymes. POR deficiency is mostly associated with Antley–Bixler syndrome (premature cranial fusion, radial synostosis, variable skeletal abnormalities and increased mortality) [39].

Testis-specific defects include 17β-hydroxysteroid dehydrogenase type 3 and 5α-reductase type 2 enzyme deficiencies. The penultimate step in testosterone synthesis is catalysed by 17β-hydroxysteroid dehydrogenase type 3 enzyme using androstenedione as substrate. The conversion of testosterone to its more active metabolite dihydrotestosterone (DHT) is catalysed by 5α-reductase type 2. Both these enzyme defects present at birth with severe undermasculinization, even normal female genitalia and/or female sex assignment in an XY infant yet a pronounced degree of virilization of the external genitalia at puberty. This phenomenon is not fully explained, other than the suggestion that peripheral production of testosterone and DHT at puberty occurs through utilization of alternative isoenzymes of the mutant enzyme.

A spectrum of mutations in the *HSD17B3* gene has been described [40]. The uterus is absent as a result of normal testicular AMH action but Wolffian ducts are stabilized, perhaps as a result of sufficient androgenic effect from locally acting high concentrations of androstenedione. Females affected with this enzyme deficiency are asymptomatic.

DHT is more potent than testosterone because it binds more avidly to the androgen receptor. Type 2 5α-reductase enzyme is expressed in the genital anlage so that growth of the genital tubercle and fusion of the labioscrotal folds is preferentially a DHT-dependent process. Mutations in the *SRD5A2* gene have been reported worldwide, often in pockets within ethnic populations. These include the Dominican Republic, where the disorder was first characterized, New Guinea, Turkey and Egypt. Affected infants usually have genital ambiguity at birth and may be sex-assigned male. Those who are raised female but virilize profoundly at puberty change gender more often than patients with other DSD conditions and patients in societies where the male and female roles are equally valued [41]. Fertility can occur following artificial reproductive techniques or even spontaneously [42].

During fetal life, DHT can be produced from 17-hydroxy-progesterone through an alternative, so-called backdoor pathway, without passing through DHEA, androstenedione or testosterone: both the classical and the alternative pathways are necessary for full virilization as shown by undervirilization in 46,XY individuals with compound heterozygous mutations in the *AKR1C2* and *AKR1C4* genes involved in the backdoor pathway [43] (Figure 4.6).

Impaired Testosterone Action

Androgen insensitivity syndrome (AIS) is a condition in which testes develop normally in a 46,XY individual and produce age-appropriate circulating concentrations of androgens that do not induce typical male development due to disruption of the androgen signalling pathway, i.e. androgen resistance. Total resistance to androgens (complete AIS, CAIS) leads to a female phenotype with bilateral testes, absence of a uterus and a short, blind vagina. Some tissue response to androgens results in the PAIS, which may manifest as mild clitoromegaly, true ambiguity of the genitalia, hypospadias alone or impaired fertility in an otherwise normal male, when it is sometimes referred to as minimal AIS (MAIS) [44]. The phenotypes of 17β-hydroxysteroid dehydrogenase and 5α-reductase deficiencies, some *NR5A1* mutations and PAIS can be so similar as to pose severe problems in diagnosis.

A mutation in the *AR* gene is identified in about 90% of XY females who have clinical, biochemical and histological evidence of CAIS but only 15–20% of patients with suspected PAIS have an *AR* mutation. The clinical outcome with regard to the need for testosterone supplementation, surgical procedures for hypospadias repair and occurrence of gynecomastia is more favourable in boys without an *AR* mutation, suggesting that resistance to androgens is not the explanation of the genital abnormality in the latter group [45]. On the other hand, low birth weight has been associated with this condition.

The pathophysiology of CAIS and PAIS is related to a defect in the intracellular action of androgens [46]

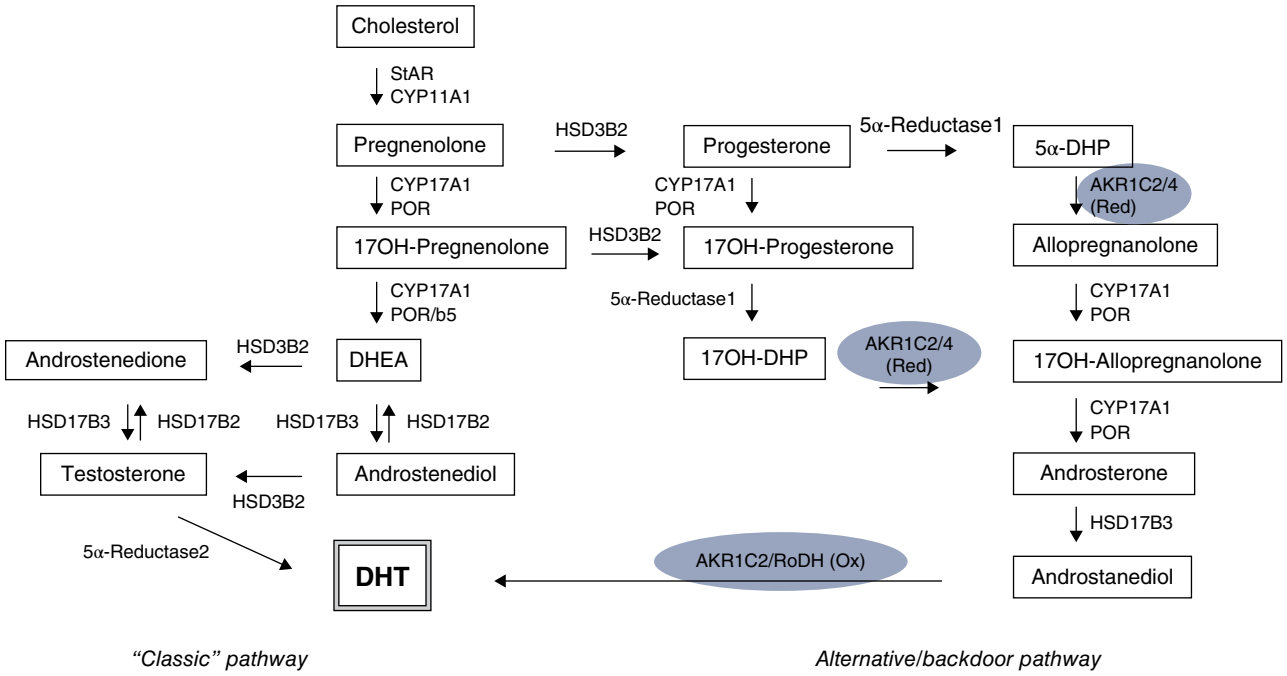

Figure 4.6 The classic pathway of steroidogenesis leading to dihydrotestosterone is shown on the left; the alternative pathway is shown on the right. The factors in the classic pathway are CYP11A1 (cholesterol side-chain cleavage enzyme, P450scc), StAR (steroidogenic acute regulatory protein), CYP17A1 (17α-hydroxylase/17,20-lyase, P450c17), HSD3B2 (3β-hydroxysteroid dehydrogenase, type 2) and HSD17B3 (17β-HSD3 [17β-hydroxysteroid dehydrogenase, type 3] and 5α-reductase, type 2 [5a-reductase 2, encoded by *SRD5A2*]). The alternative pathway is characterized by the presence of additional enzymes: 5α-reductase, type 1 (5a-reductase 1, encoded by SRD5A1), AKR1C2 3 (3α-reductase, type 3) and possibly AKR1C4 (3α-reductase, type 1) and RoDH (3-hydroxyepimerase, encoded by *HSD17B6*).

(Figure 4.7). The AR is located in the cytoplasm of androgen target cells complexed to heat shock proteins until bound to testosterone or DHT, when the hormone receptor complex translocates to the nucleus. Acting as a transcription factor, this complex binds as a homodimer together with co-regulator proteins to promote expression of androgen-responsive genes. It is possible to postulate a number of steps in this pathway that could result in resistance to androgens, such as defects in androgen binding or disrupted interaction of the hormone receptor complex with DNA, but evidence for these mechanisms is lacking.

The *AR* gene is located on chromosome Xq11-12. More than 1000 mutations that cause either CAIS or PAIS have been identified in this 90 kb gene, which are recorded on an international database (http://androgendb.mcgill.ca/). Severe mutations, such as deletions and premature stop codons, predictably result in no AR function and a CAIS phenotype but most mutations are missense and located in the ligand-binding domain. The same mutation may cause CAIS in one family but be manifest as PAIS in another.

The factors modulating receptor activity and androgen responsiveness that result in phenotype variability are unclear but may include somatic mosaicism and variation in AR trinucleotide repeat lengths. The AR contains a polymorphic trinucleotide CAG repeat that encodes a polyglutamine tract and, further downstream in the N-terminal domain, a polymorphic trinucleotide GGN repeat encodes a polyglycine tract. The range of repeats for both tracts in the normal population is about 10–35.

The polyglutamine tract is expanded in spinal and bulbar muscular atrophy (SBMA, Kennedy disease) and affected males display signs of mild androgen insensitivity. Transcriptional efficiency of the AR *in vitro* is inversely proportional to the number of CAG repeats but *in vivo* weaker AR activity appears to be compensated by higher testosterone concentrations. Nevertheless, the actual number of CAG repeats appears to be biologically relevant since numbers above or below the average of 22–23 have been associated with increased risk for infertility and variations in the number of CAG repeats within the normal range show associations with several androgen-related disorders such as prostate cancer, ovarian hyperandrogenism, androgenic alopecia and severity of coronary artery disease. The biological relevance of the GGN repeat is less clear [46].

Little is known about the identity of androgen-responsive genes expressed in the developing male reproductive tract. Apolipoprotein D (APOD) has been identified as such an AR target gene and is upregulated in genital skin fibroblast cultures following DHT treatment. Thus

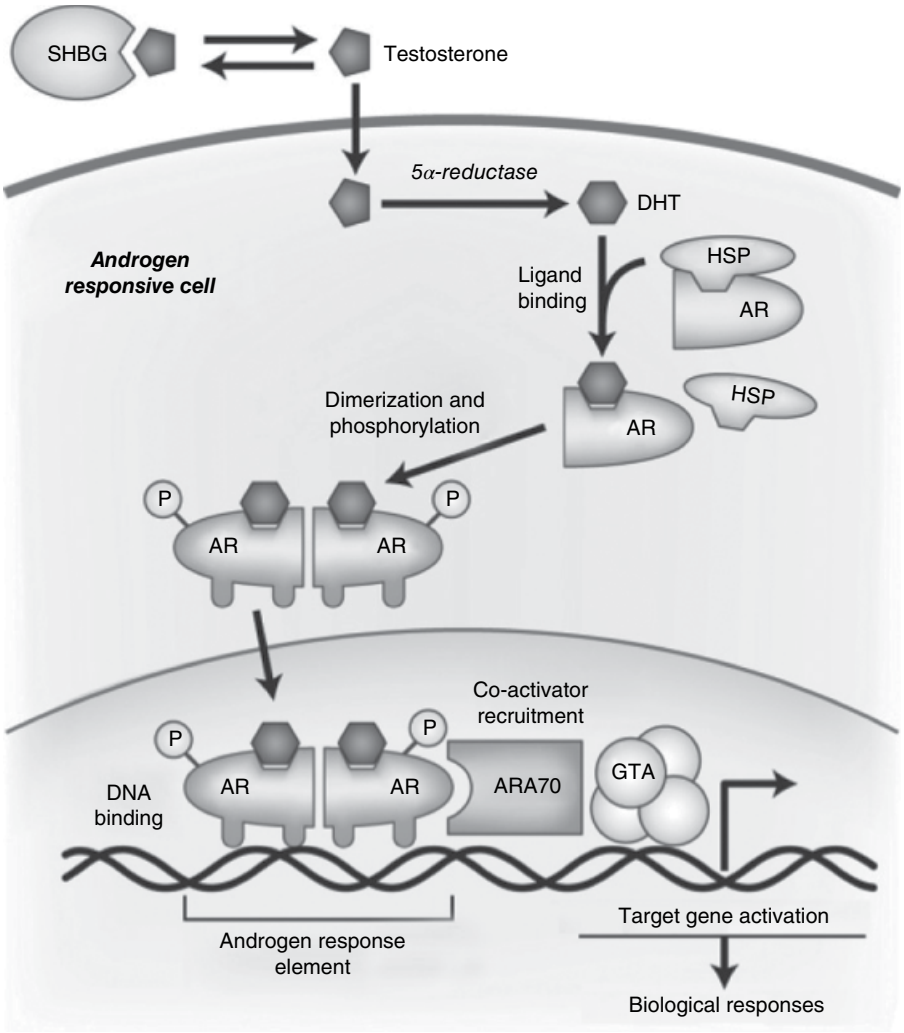

Figure 4.7 A schematic diagram of androgen action in a target cell. Circulating testosterone bound predominantly to sex hormone-binding globulin (SHBG) enters the cell in free form where it is converted to dihydrotestosterone (DHT), a more potent androgen. Both androgens bind to a single cytoplasmic androgen receptor (AR) complexed to heat shock proteins (HSPs) and other co-chaperones such as FKBP52. Androgen binding dissociates the AR from HSPs, where AR-bound androgen translocates to the nucleus, binding to DNA response elements as a homodimer. Coactivators, such as ARA70, bind to the AR complex to mediate interaction with the general transcription apparatus (GTA). This results in transcription of androgen-responsive genes and pleiotropic biological responses; examples of such responses would include male sex differentiation, growth, muscle and bone development, spermatogenesis and prostate growth. P, phosphorylation. *Source:* From Hughes and Achermann [1], with permission.

quantification of APOD induction in labioscrotal skin fibroblasts of patients with presumed AIS in whom no AR mutation can be identified is a useful alternative way to assess AR function and confirm a diagnosis of AIS [47]. In addition, AR signalling has been shown to induce epigenetic changes in genomic DNA derived from genital tissues [48].

Virilization of an XX Individual

Ninety-five percent of 46,XX newborns with atypical genitalia are individuals with CAH due to 21-hydroxylase deficiency; other causes of virilization in XX individuals are rare. The placenta contains an aromatase enzyme system that is generally extremely efficient in protecting a female fetus from the effects of androgens in the maternal circulation but androgen-secreting tumours of the adrenals and ovaries can masculinize the mother and her female fetus, presumably because the androgen substrates overwhelm the placental aromatase system. Luteomas of pregnancy are benign tumours but produce large ovarian masses. Luteomas predominate in multiparous Afro-Caribbean women who may have pre-existing polycystic ovary syndrome. The tumours regress postpartum but can recur in subsequent pregnancies. Other virilizing ovarian tumours include arrhenoblastoma, hilar cell tumour and Krukenberg tumour.

The use of progestational agents with some androgenic activity to prevent recurrent miscarriage is obsolete but danazol, a derivative of 17β-ethinyltestosterone, has a place in the medical treatment of endometriosis. It readily crosses the placenta and cases of masculinized female infants have been recorded [49].

Placental aromatase deficiency is a recognized cause of ambiguous genitalia in a female infant whose mother may also be virilized during pregnancy [50]. A single *CYP19* gene is expressed through the action of tissue-specific promoters in several tissues, including the gonads, placenta and adipocytes. The aromatase enzyme is a key regulator of production of oestrogens from androgens in the feto-placental-maternal unit (Figure 4.8). The fetal adrenals produce large quantities of dehydroepiandrosterone sulphate (DHEAS) that is 16β-hydroxylated in both the fetal adrenal and liver. After transfer to the placenta, the sulphate moiety of 16OH-DHEAS is removed by placental sulphatase. DHEA and 16OH-DHEA are converted to more potent androgens, such as androstenedione and testosterone, which are aromatized to oestrone and oestradiol, respectively. A large amount of oestriol is also produced by aromatization of androgen substrates. Serial measurements of urinary oestriol have a useful role in monitoring prenatal treatment of CAH with dexamethasone [51]. The degree of maternal and fetal masculinization can be quite profound in placental aromatase deficiency but the mother may escape signs of virilization when as little as 1–2% activity of mutant enzyme is present. This illustrates the capacity of this enzyme to convert androgens to oestrogens. The internal genitalia of affected female infants are normal but ovarian cysts may develop in later childhood. At puberty, there is failure of breast development, virilization and polycystic changes in the ovaries. A spectrum of mutations is distributed throughout the *CYP19* gene. Aromatase deficiency should be considered when CAH has been excluded in a female newborn with ambiguous genitalia.

Combined deficiency of the P450 17α-hydroxylase and 21-hydroxylase enzymes can also cause mild degrees of maternal and fetal masculinization that is self-limiting after birth. Mutations are found not in the *CYP17* or *CYP21A2* genes but in the gene encoding for cytochrome POR [52, 53]. This enzyme functions as an electron donor to microsomal cytochrome P450s. This may partly explain the masculinization of female affected infants from accumulation of fetal adrenal androgens as a result of partial placental aromatase deficiency but there is also impairment of androgen biosynthesis in oxidoreductase deficiency such that affected males are undermasculinized.

The explanation for this paradox has been attributed to the existence of the 'backdoor' pathway, in which the potent androgen DHT can be produced from the precursor steroids 17-hydroxyprogesterone and androstanediol and avoiding testosterone as an intermediary substrate. This pathway exists in the human fetus only to switch to the more classic pathway of androgen biosynthesis after birth (Figure 4.6) [43]. It is hypothesized that the difference in virilization seen in 46,XX infants with 21-hydroxylase defects as compared with 3β-hydroxysteroid dehydrogenase defects is related to the existence of this alternative pathway, since DHT will be produced in the former but not in the latter condition. Most patients with POR deficiency have the skeletal malformations characteristic of the Antley–Bixler syndrome.

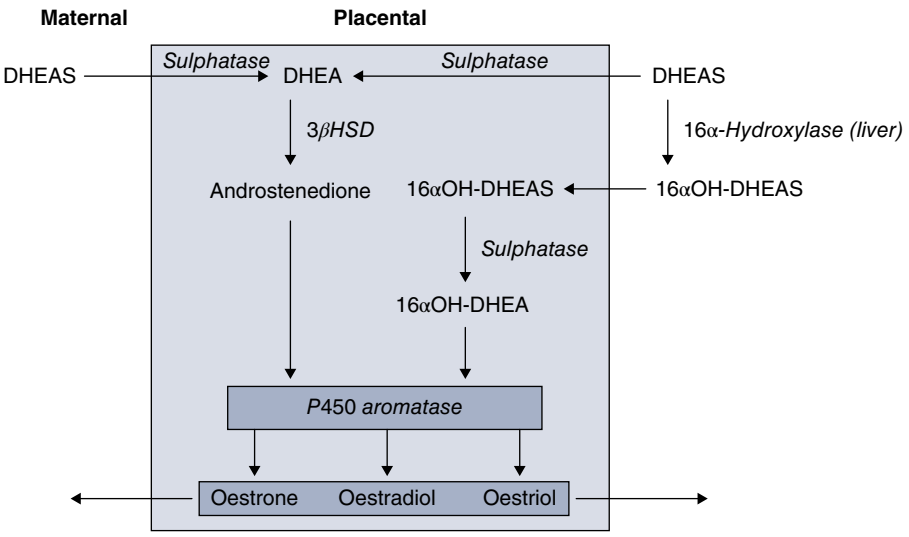

Figure 4.8 The feto-placental-maternal steroid unit. The androgen substrate DHEAS is synthesized in both the maternal and fetal adrenals and cleaved to DHEA by placental sulphatase. The fetal liver also hydroxylates DHEAS prior to sulphatase cleavage by the placenta. Androgen substrates are aromatized to oestrogens, particularly oestriol. DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulphate; 3βHSD, 3β-hydroxysteroid dehydrogenase.

Biallelic mutations in the 3-prime-phosphoadenosine 5-prime-phosphosulphate synthase 2 (PAPSS2), disrupting the sulphation of DHEA to DHEAS, have also been shown to cause increased androgen concentrations in girls. This condition seems to result in premature pubarche and hyperandrogenic anovulation rather than atypical genitalia [54].

In the context of ambiguous genitalia, CAH due to 21-hydroxylase deficiency is the most common cause and the most straightforward to diagnose. This must be undertaken promptly in view of the potential life-threatening consequences of glucocorticoid and mineralocorticoid deficiencies to the infant. Giving dexamethasone to the mother from early in pregnancy can successfully prevent masculinization of the external genitalia in an affected female infant. This practice is hotly debated because seven unaffected fetuses are exposed unnecessarily to high doses of glucocorticoids and possible impairment of brain function for the treatment of one physical consequence in the affected fetus, since the disease itself is not affected by this therapy [55].

Management of DSD

Table 4.2 lists problems in a newborn infant or during childhood that merit investigation for a possible DSD. The frequency of deviation of the genital anatomy from the typical male or female newborn is estimated to be as high as 2% of live births, which includes sex chromosome aneuploidies, distal hypospadias and unilateral undescended testis [56].

Table 4.2 Newborn problems or situations in childhood that merit workup for DSD.

Ambiguous genitalia	
Apparent female genitalia with	Enlarged clitoris
	Posterior labial fusion
	Inguinal/labial mass
Apparent male genitalia with	Bilateral undescended or non-palpable testes
	Isolated proximal hypospadias
	Isolated microphallus
	Distal or mid-shaft hypospadias with undescended testes
	At least one undescended testis and one of the above, including familial subfertility
Family history of DSD	For example, CAIS, risk of POF in case of NR5A1 mutation
Genital anomalies associated with syndromes	
Discordance between genital appearance and prenatal karyotype	

Anthropometric measurements, such as penile and clitoral length and width and testicular volumes, differ among populations and ethnic variations should be taken into account when evaluating a possible DSD. The anogenital distance has been proposed as a useful marker to reflect androgen action during early fetal life, which may also correlate with outcome parameters such as behaviour and reproductive potential in males [57]. No consensus has been reached about standardized assessment of anogenital distance and measuring it in babies is subject to considerable inter-observer variability. There are reference data available for measurements of the external genitalia in women, such as clitoral size, labial length and length of perineum [58]. This is important information when planning reconstructive surgery in disorders such as CAH.

An International DSD Registry exists (www.i-dsd.org) where patients can be registered by healthcare professionals in a secure virtual research environment, provided that appropriate consent has been obtained. The I-DSD Registry facilitates clinical and translational research on a global scale and contains data on over 3000 individuals.

Initial Evaluation and Diagnostic Approach

After administration of urgent medical care and appropriate parental support, all neonates, children, adolescents and older individuals with unexpected sexual characteristics should be referred to a hospital with a multidisciplinary DSD team. Such a team requires a paediatric endocrinologist, paediatric urologist and child psychologist with specific expertise in DSD. Additional team members may include geneticists, neonatologists, gynaecologists, specialized nurses, social workers and ethicists depending on local circumstances. Special consideration should be given to an unbiased approach to the decision on sex of rearing and the use of a non-binary and non-stigmatizing vocabulary to describe the external genitalia and/or the condition. Terms such as 'normal' and 'abnormal' can be replaced by more neutral terms such as '(a)typical' or 'variant'; the term 'intersex' has been replaced by 'DSD' for 'disorders' or preferably 'differences of sex development'. Adequate support from hospital staff may be of great long-term value for increasing parent–child bonding and acceptance of the condition. The DSD team should ensure that the needs of parents and affected persons regarding comprehensive information and psychological support are fully and timely addressed [59].

Examination

Family history and exposure to potential reproductive tract teratogens are relevant next to a physical examination. A number of grading systems have been devised to describe genitalia in specific conditions such as AIS [60], the degree of virilization in female infants with CAH [61] or in male undermasculinized infants [62]. The latter assigns a score in relation to the presence or absence of micropenis, the position of the urethral opening (normal, glandular, penile shaft, perineal), whether or not the scrotal sac is fused and the position of the gonads (scrotal, inguinal, abdominal, absent). A modified version of this scoring system that can be applied to all infants, irrespective of their sex of rearing, is currently being validated.

A value of 2.5 cm or less for stretched penile length, corresponding approximately to −2 SD, is often used as a cut-off for short penile length and indicates a need for further investigations, although it may be necessary to consider ethnic variations [63]. A normal range for penile length in preterm infants 24–36 weeks' gestation is available [64]. Applying a quantitative system to characterize the degree of undervirilization in XY infants seems desirable, particularly if treatment with androgens is to be tried. The remainder of the assessment should include evidence of adrenal insufficiency and consideration of any congenital malformation syndromes associated with genital anomalies.

Hormonal Workup

Since the cause of ambiguous genitalia at birth is CAH due to 21-hydroxylase deficiency in up to 95% of neonates, this condition should be excluded before other investigations are undertaken.

Many schedules have been suggested for the investigation of a newborn infant with ambiguous genitalia but each unit must formulate a protocol determined by local practice and facilities [65]. Many centres are equipped to perform screening investigations but more detailed investigations to establish a definitive diagnosis may have to be undertaken elsewhere. Plasma or serum steroid profiling should be performed in specialized laboratories, using combined chromatography and mass spectrometry-based methods [5]. Table 4.3 lists a range of investigations that should lead to a functional diagnosis in most newborns with ambiguous genitalia and allow an early decision regarding sex of rearing.

In reality, the leading causes are CAH, *NR5A1* and *AR* mutations and X/XY mosaicism. The karyotype result will indicate the category of DSD according to the classification outlined in Table 4.1 and will guide further workup. A provisional indication of the sex chromosomes can be obtained rapidly by FISH or qPCR with X- and Y-specific probes. A full karyotype is required to confirm the FISH or qPCR result and a sufficient number of mitoses must be analysed to exclude mosaicism.

The majority of 46,XX DSD cases are caused by different forms of CAH, which can be diagnosed using a specific urine or plasma steroid profile [5]. Measurement of serum 17OH-progesterone (invariably >300 nmol/L) and a uterus visualized on pelvic ultrasound is a reliable test for 21-hydroxylase deficiency CAH in a 46XX infant with ambiguous genitalia. Ancillary biochemical tests should establish whether the infant is also a salt-loser, although mineralocorticoid replacement therapy should be started in all forms of classic CAH [67].

It may be necessary to perform an ACTH stimulation test if CAH is suspected in a preterm infant or if there is a possibility of one of the rarer enzyme defects. Measurement of urinary steroid metabolites by gas chromatography and mass spectrometry is the definitive test to define the type of enzyme defect in all forms of CAH [5, 68]. All male-looking infants with impalpable testes must have immediate karyotype and hormonal investigations to exclude a diagnosis of 21-hydroxylase deficiency.

Hormone concentrations in CAIS and PAIS are consistent with the definition of target resistance; testosterone is markedly elevated and LH concentrations are not suppressed. Androgens are aromatized to oestrogens resulting in breast development. Thus, girls with CAIS are undistinguishable from their peers in early puberty except for absent or scanty pubic and axillary hair. Clinical presentation typically occurs for assessment of primary amenorrhoea.

Occasionally, the condition may present in infancy with inguinal herniae that are found to contain testes at the time of surgical repair so a karyotype should be performed in female infants with an inguinal hernia. 1.1% of premenstrual girls with inguinal herniae have CAIS, which may also be detected from a mismatch between prenatal sexing and birth outcome. Gynaecomastia in a boy with a history of hypospadias or undescended testes should exclude PAIS.

A diagnosis of (ovo)testicular DSD is suspected in a child with a 46,XX karyotype, ambiguous or typical male genitalia and normal steroid profiles but with concentrations of AMH and testosterone above the female range. A (hemi)uterus may be present. FISH or PCR for *SRY* identifies a translocation of *SRY* to the X chromosome or one of the autosomes, as is mostly seen in 46,XX individuals with typical male genitalia. In patients with *SRY*-negative XX (ovo)testicular DSD, further genetic testing includes high-resolution array CGH and/or targeted copy number analysis (e.g. by multiplex ligation-dependent probe amplification or MLPA) to detect SOX9 duplications, genomic rearrangements of the *SOX3* region or

Table 4.3 Investigating an infant with a suspected DSD.

Genetic tests	Remarks
FISH	Locus specific, detects large CNVs such as deletions or duplications, high number of counted cells necessary to exclude mosaicism
qPCR	Gene specific, detects subtle CNVs (deletions, duplications) up to exon level
Karyotype	Allows detection of numerical (aneuploidy and polyploidy) and structural chromosomal anomalies (such as reciprocal and Robertsonian translocations, inversions) and of mosaicism, second tissue useful in case of suspicion of mosaicism
MLPA	Gene specific, detects subtle CNVs (deletions, duplications) up to exon level, methylation defects
Array CGH	Genome-wide, detects CNVs, mosaicism
Sanger sequencing	Gene specific, detects SNVs and small insertions and deletions, confirmation of sequence variants identified by WES
Targeted NGS	Large panels of relevant genes, detects SNVs and (sometimes) CNVs, bioinformatic pipeline needed, limited risk of incidental findings
WES and WGS	Genome-wide, detects SNVs and structural variations including CNVs and balanced rearrangements, allows identification of new genes and genetic pathways in research setting, extensive bioinformatic pipeline needed, risk for incidental findings (ethical issues)
Endocrine and others	
17OH-progesterone, 11-deoxycortisol, 170H-pregnenolone, DHEA, renin, ACTH	Consider Synacthen test in case of unclear diagnosis and suspicion of CAH, analysis preferably performed by liquid chromatography and mass spectrometry
24 hours of urinary steroids (or urinary spot)	Analysis performed by gas chromatography and mass spectrometry
Proteinuria	If positive, check for WT1 mutation (Frasier syndrome)
Testosterone androstenedione, DHT	HCG test (choice of protocol in function of age) outside the postnatal gonadotropin surge period
LH, FSH, AMH, inhibin B	Interpretation of gonadotropins in function of age and pubertal stage
Imaging	
Pelvic, adrenal, cardiac/renal US	Visualization of the uterus may be difficult, large adrenals suggestive of adrenal hyperplasia, exclude Wilms' tumour in case of possible WT1 mutation, cardiac and renal US in case of 45,X/46,XY
Surgical	
Laparoscopy	Visualization of internal anatomy, gonadal biopsy in case of suspicion of gonadal dysgenesis and/or abdominal gonads
Gonadal biopsy	Immunohistochemical staining with specific markers of gonadal development (OCT3/4, DDX4/TSPY/VASA, SOX9, FOXL2, KITLG) necessary, highly informative in case of expert evaluation
Genital skin biopsy	Gene expression studies

Source: Modified from Achermann et al. [66].
FISH, fluorescent in situ hybridization; CNVs, copy number variations; qPCR, quantitative polymerase chain reaction; MLPA, multiplex ligation-dependent probe amplification; array CHG, microarray comparative genomic hybridization; SNVs, single nucleotide variations; WES, whole exome sequencing; NGS, next-generation sequencing; WGS, whole genome sequencing; CAH, congenital adrenal hyperplasia; WT1, Wilms' tumour 1; DHT, dihydrotestosterone; hCG, human chorionic gonadotropin; US, ultrasound.

copy number variations of *SOX10*. Sequencing of NR5A1 to exclude presence of the p.R92W variant is indicated. In the presence of skin abnormalities, *RSPO1* mutations must be excluded. Targeted next-generation sequencing or whole exome sequencing can be performed in unsolved cases.

In an XY or X/XY infant with ambiguous genitalia, investigations are aimed at establishing the presence and function of testicular tissue. Assessment of Sertoli cell function is pivotal in this situation and based on measurement of AMH and/or inhibin B. AMH and inhibin B concentrations are not greatly influenced by the postnatal gonadotropin surge, sometimes referred to as 'mini-puberty'. High (i.e. within the male reference range) circulating concentrations of AMH/inhibin B indicate testis development with good Sertoli cell function

suggestive of a steroidogenic disorder or AIS; low concentrations are found in partial gonadal dysgenesis and an undetectable or very low value suggests anorchia in association with a male phenotype or complete gonadal dysgenesis in case of a female phenotype [20, 69]. Gonadotropin concentrations are elevated during the postnatal surge or in puberty but may be unremarkable outside these periods. Absence of the postnatal gonadotropin surge is suggestive of hypogonadotropic hypogonadism but has also been documented in AIS [23, 70].

Outside the mini-puberty or true puberty periods, hCG stimulation is required to assess Leydig cell function. There is no uniform protocol for this test. In neonates and infants, a single injection (1500 units IM) with a post-hCG blood sample collected 72 hours later is often sufficient. A more cumbersome but more reliable alternative in older children is 1500 units/day for 3 consecutive days with blood sampling 24 hours after the last injection. Occasionally, a longer test is needed using a twice weekly injection regimen for 3 weeks [71]. Pre- and post-hCG blood samples should be analysed for androstenedione, testosterone and DHT.

Expressing the ratio of testosterone to androstenedione and of testosterone to DHT following hCG is a useful screen for 17β-hydroxysteroid dehydrogenase and 5α-reductase deficiencies, respectively, in XY DSD. Nevertheless, differentiating these conditions, *NR5A1* mutations and PAIS on the basis of hormonal data, remains challenging and certainty can be obtained only by molecular studies [69]. When combined adrenal and testicular steroidogenic disorders are suspected, urine and/or serum steroid profiling based on chromatography and mass spectrometry, probably following ACTH stimulation, can confirm the diagnosis [5].

Imaging with ultrasound and magnetic resonance imaging (MRI) is used to delineate internal genital anatomy, including localizing the urogenital confluence and the site and, possibly, the morphological nature of the gonads. A small uterus can be difficult to visualize [72]. Only histology will provide details of the gonadal differentiation pattern(s) and many infants with ambiguous genitalia require a laparoscopy to reach a diagnosis.

Genetic Tests

Obtaining a specific diagnosis can be important to exclude involvement of other organ systems (e.g. aortic dilation in 45,X/46,XY, renal failure in Frasier syndrome) or identify possible life-threatening associated conditions (e.g. acute adrenal crisis in CAH, Wilms' tumour in Denys–Drash syndrome). Such a diagnosis will also facilitate genetic counselling and guide management with regard to sex of rearing and possible genetic profiling of embryos before implantation in assisted reproduction. In spite of current possibilities for large-scale genomic approaches, careful selection of the most appropriate genetic test for each condition remains the cornerstone of good clinical practice [66]. An overview of frequently used genetic tests and their most important characteristics is provided in Table 4.3.

The presence of minor Turner stigmata, such as short fourth metacarpal/metatarsal or short stature in an individual with presumed 46,XY gonadal dysgenesis, should always prompt investigations to exclude 45,X/46,XY mosaicism. FISH with X and Y centromere probes in at least 100–200 interphase cells from 2 different tissues (e.g. peripheral blood and a buccal smear or skin fibroblasts or directly on gonadal tissue) can identify low-grade mosaicism.

Candidate gene Sanger sequencing is most appropriate where phenotypic, biochemical or family data suggest a specific single-gene cause [66]. Unexplained proteinuria in a boy with a history of hypospadias or in an adolescent girl with primary amenorrhoea should prompt analysis of the *WT1* gene. A family history of POI is suggestive of *NR5A1* mutations. Primary adrenal insufficiency in association with 46,XY DSD may point to rare homozygous *NR5A1* mutations or an early block in steroidogenesis (e.g. due to mutations in *STAR*, *CYP11A1, HSD3B2*, *POR*). Congenital heart defects in association with 46,XY partial gonadal dysgenesis have been found in individuals with *GATA4* defects.

Array comparative genomic hybridization is useful as a first-line approach in children with complex phenotypes [69]. In cases where many differential diagnoses based on phenotypic and hormonal data are possible, clinical high-throughput tests based on next-generation sequencing methods with a panel of known DSD-causing genes may allow a molecular diagnosis in up to 30% of cases [73]. Whole exome and whole genome approaches are mostly applied in a research setting; difficulties with data filtering and interpretation as well as ethical considerations regarding generation of 'bystander information' (i.e. susceptibility for unrelated genetically determined conditions) hinder their large-scale implementation in diagnosis [66].

Sex Assignment, Gender Development, Early Genital Surgery and Disclosure of the Condition

Although most individuals with a DSD have no gender issues, gender related distress can be present in partial gonadal dysgenesis, PAIS and other forms of DSD. Individuals with complete gonadal dysgenesis or CAIS typically have a female gender identity. Although most 46,XX individuals with an early diagnosis of CAH identify as females and sex assignment in countries with an

established neonatal screening programme in general accords with this, many individuals living in the male gender have been described and this may be the most frequent situation in countries without such a screening programme [74–76].

In 5α-reductase deficiency, gender role in those raised female changes in up to 60%, depending largely on the societal context. Such gender role changes are not so common in 17β-hydroxysteroid dehydrogenase deficiency, which is also characterized by a predominant female phenotype at birth and profound virilization at puberty [41].

Many political activists and patient advocacy groups firmly oppose a dichotomous view on sex and gender and medical professionals are increasingly open to their arguments. However, although some have voiced opinions against any decision regarding sex of rearing at birth, there still seems to be a general consensus that leaving the sex of a child undetermined for many years may not contribute to the best outcome [6]. This decision in some DSD cases can be extremely challenging: information imparted to the parents at this early stage must be accurate and based on fact and not speculation because misinformation may be a lifelong burden for the family.

A decision on sex of rearing may not be possible until many investigations have been completed. The decision is based primarily on the gender identity to be expected later from the results of all investigations, rather than on the karyotype, the degree of genital ambiguity, the need for and availability of specialized genital surgery or the presence or absence of testes or a uterus alone. Societal and religious issues and perspectives with regard to hormonal and sexual function must also be taken into account. The decision is multidisciplinary and should meet parental expectations. More than in the past, a male sex is assigned in cases of antenatal androgen exposure. Early and age-appropriate disclosure of the condition to the child is paramount [77–79].

Genital surgery with the aim of normalizing genital appearance in childhood is increasingly controversial and should be performed with great caution and after having explored, together with the parents, all options. Rarely would surgery now be undertaken for less than Prader stage III clitoral hypertrophy in girls with CAH. This trend has been influenced by outcome studies in adult CAH women who underwent feminizing cosmetic genitoplasty and who subsequently have impaired genital sensitivity with adverse effects on sexual function [80]. Consultation with a child psychologist and/or other mental healthcare workers is as important as seeking surgical advice [81]. If possible, decisions regarding genital surgery should be postponed until the child can contribute to the decision but medically indicated surgery to avoid or treat complications (e.g. fistula repair) should not be delayed. Genital surgery should be performed only by a specialized team and after appropriately obtaining informed consent [82].

Early disclosure is paramount for shared decision-making and enables the individual and family to live with the condition. Early diagnosis allows the child to 'grow into disclosure' guided by his/her parents and the physician, taking important developmental milestones into account [83].

Clinical Management

Once the diagnostic phase has been completed and decisions regarding sex of rearing and need for early surgery have been taken, management hinges on the condition and is tailored on the needs and expectations of each individual.

Throughout childhood, parents and children need to be informed about readily available access to expert medical evaluation, psychological support and help with the disclosure process. In older children who have a condition with unclear gender outcome, formal assessment of gender identity should precede decisions about gonadectomy and/or start of pubertal induction.

There is some evidence that a short course of testosterone given during infancy (e.g. Sustanon® 25 mg/month IM for 3–6 months) can increase penile size but whether this will result in increased adult penile length is not known. Some adult men with penile insufficiency may benefit from phalloplastic surgery but, given the complexity and impact of this procedure, counselling of candidates and thoughtful consideration of all other therapeutic options is imperative [84].

Schedules for follow-up of gonadal function and pubertal induction generally follow those for other conditions associated with primary gonadal failure. Although some data are available for specific conditions such as CAH and CAIS [85, 86], long-term outcome data with regard to general health, metabolic outcome or bone health are generally lacking. Possible interventions in preparation of later assisted reproduction procedures, such as gonadal/germ cell cryopreservation, should be discussed if appropriate. Future clinical applications resulting from cellular reprogramming techniques will probably be relevant for many children living with a DSD today.

Girls with a short vagina need to be informed at an appropriate time about possibilities for vaginal reconstruction. Self-dilatation with support from a dedicated team consisting of a psychologist, gynaecologist and physical therapist is successful as first-line therapy in up to 80% of cases. Forced dilatation in a hospital

setting ('Vecchietti procedure') may be a valuable alternative for girls who have difficulties in complying with self-dilatation [87]. Some experience of uterus transplantation is available [88].

Gonadal Tumour Risk and Indications for Gonadectomy

Gonadectomy can be performed because of gonadal tumour risk, hormone production discordant with gender identity or both. Germ cell tumours occur with increased incidence in 46,XY and 45,X/46,XY DSD but the risk is highly dependent on the specific condition. Conditions associated with gonadal dysgenesis have a much greater risk for developing a germ cell tumour than conditions that are due to hormonal defects; in addition there appears to be a correlation with the degree of gonadal differentiation, such that conditions causing an early block in gonadal development such as *SRY* or *WT1* mutations have the highest risk.

Other factors influencing tumour risk are the presence of androgens (with tumour risk strongly increasing after puberty), initial location of the gonad (a lower risk is expected in scrotal as compared with inguinal or abdominal gonads) and age. The peak incidence follows that of germ cell tumour occurrence in the general male population, which occurs in early adulthood. Invasive germ cell tumours, mostly seminomas, are preceded by premalignant changes, termed germ cell neoplasia *in situ* (GCNIS), for many years. GCNIS may present as carcinoma *in situ* of the testis or gonadoblastoma of the dysgenetic gonad; these early neoplastic changes are subtle and require expert evaluation and specialized immunohistochemistry to distinguish them from benign changes such as maturational delay of germ cells. Due to confusion with the latter situation, the incidence of germ cell tumours occurring in DSD patients has been overestimated in the past, especially in individuals with disorders of testosterone biosynthesis or action. Markers that have been found informative in this context are pluripotency markers such as OCT3/4 and NANOG; germ cell markers like TSPY, VASA or DDX4; markers specific of male or female gonadal development, such as SOX9 and FOXL2, respectively; and KITLG, which is crucial to discriminate maturation delay of germ cells from early neoplasia.

In cases with non-functional gonads and high (30–50%) tumour risk, such as 46,XY girls with complete gonadal dysgenesis, prophylactic gonadectomy is generally performed as an elective surgical procedure following the diagnosis. In many cases with partial gonadal dysgenesis, Leydig cell function seems to be better preserved than Sertoli cell function, resulting in variable degrees of virilization during puberty and warranting gonadectomy before puberty in affected girls. Excessive pubertal virilization has been described in some cases with *NR5A1* mutations [14].

A decision regarding prophylactic gonadectomy in boys with partial gonadal dysgenesis who have a considerable risk for germ cell tumour development on the one hand but may benefit from endogenous hormone production on the other can be extremely difficult; repeat gonadal biopsies and expert evaluation to detect early malignant changes can be informative.

No reliable data exist on tumour risk in testosterone biosynthetic disorders and PAIS but the risk is thought to be much lower than in gonadal dysgenesis. Lack of testosterone action may have a protective effect. Gonadectomy is mostly performed before puberty in affected girls with Leydig cell hypoplasia, PAIS, 17β-hydroxysteroid dehydrogenase deficiency or 5α-reductase deficiency in order to avoid virilization. In the latter two conditions, impressive virilization has been observed at puberty, which is mostly attributed to activation of alternative isoenzymes. Regular self-examination and annual testicular ultrasound from puberty onwards are advised in boys living with these conditions. A single testicular biopsy in late adolescence may be informative about the presence of extensive GCNIS [89, 90]. In cases with unclear gender identity, GnRH analogues have occasionally been applied in order to temporarily suppress endogenous hormone production.

There is no consensus about the need for gonadectomy in CAIS and the procedure should be discussed in young adulthood. Most data point to a risk of GCNIS in about 10% of adult CAIS women but epidemiological evidence suggests that most of these lesions will not be invasive. Some adults with CAIS opt not to undergo gonadectomy in view of the low tumour risk and benefits from endogenously produced oestrogens (through aromatization) or because they consider the presence of androgens conducive to improved well-being [91]. Some of those who have had earlier gonadectomy have requested androgen replacement.

The mechanism of this effect is biologically difficult to reconcile if one accepts the dogma that all androgens mediate their effect by ligand activation of a single AR ubiquitously expressed throughout the body, including the brain. None of the existing tumour markers (PLAP, β-hCG) reliably detect seminomas, the prevailing tumours in CAIS, so, for those adults who opt not to have gonadectomy, monitoring for tumour development has to rely on MRI and ultrasound, neither of which are sufficiently informative for abdominally located gonads. Screening methods, such as detecting tumour-specific microRNAs or genetic profiling of germ cell cancer susceptibility alleles, are being evaluated [92].

Information and Support for Parents Having a Baby with DSD

The first question parents having a baby are faced with is: is it a girl or a boy? If this question cannot be answered clearly, this creates severe distress.

Giving comprehensive medical information and psychosocial support are crucial in that vulnerable period. Stress, post-traumatic stress disorder (PTSD) and a high need of psychological support were reported by families having a child with a DSD [93]. Not understanding and confusing information were the only factors predicting stress and PTSD [94, 95]. Usually the complex medical information is given to the parents by a DSD-specialized pediatric endocrinologist. It is important that the information is communicated in a comprehensive and sensitive manner at several visits after diagnosis but the family should be encouraged to accept psychological support, the major aim of which is to instil bonding with a child with atypical genitalia. The issues of DSD as a chronic condition involving later identity, sexuality and fertility should be addressed in a sensitive manner.

An important aspect of psychological counselling is the need for parents to experience for themselves what sex and gender means to them. This enables them to understand that immediate surgical referral of the child does not resolve the issue of ambiguous genitalia associated with DSD. This is essential for awareness and acceptance of the child's individuality and to support his/her development throughout childhood according to his/her individual needs. The process may take considerable time. Contact with other families who have a child with a DSD can be helpful and offered through support groups, information brochures and websites, e.g. www.dsdfamilies.org. Peer counselling, which is available in some countries, is recommended.

Information and Support for Children and Adolescents

When the diagnosis is made in childhood or puberty, the child or adolescent has to be involved in the process of information management and decision-making in an age-appropriate manner. Adults with a DSD report that the former practice of non-disclosure of the diagnosis and not having been involved in decisions concerning medical procedures was most traumatizing for them. A psychologist can be of great value in age-appropriate disclosure of the condition to the child and adolescent by helping the child share information with the social environment, by raising awareness of gender variations and by discussing gender role behaviour and in the decision-making process with regard to medical interventions such as hormone therapy and surgery. Psychological counselling is especially needed when specific milestones are reached, such as beginning of day care, school and puberty, and should be offered to the families and child according to their individual needs. Contact and exchange with other families or peers from support groups can be extremely helpful but there are many ways to handle a DSD during a lifetime and each family and child or adolescent has to find the way which fits best with their personal situation [83].

Transition

The transition from puberty to early adulthood is a time when many decisions on future life have to be taken by the adolescent and young adult (AYA). AYAs need to develop autonomy and learn to balance occupational, academic, familial, financial, personal and romantic demands on their own.

AYAs with chronic conditions have to become familiar with self-management of their condition. At this time they should be informed about general issues such as health behaviour, sexuality and mental health. AYAs with a DSD have to learn specifically about their hormone therapy, body image, fertility issues and possible genital surgery. They should know about effects and side effects of hormone therapy. The impact of DSD on sexuality and variations in sexual orientation should be addressed. A DSD-specific checklist facilitating communication around transition from pediatric to adult care has been developed (Table 4.4) [96].

In many patients with atypical genital development, genitoplasty or vaginal dilatation may be necessary. These procedures are often performed during transition, a vulnerable phase in which AYAs shape their identity and must gain sexual confidence. The AYA should be informed that appropriate follow-up by a specialized gynaecologist/urologist is necessary after genital surgery. A sexual therapist should be involved. The AYA is often confronted with the challenge of infertility for the first time. It is important to explain all actual and future options for assisted reproduction technologies.

Psychological support can be very helpful in the transition phase to empower the AYA to handle these burdensome issues in addition to his or her chronic condition; refusal of the AYA to accept such support is frequent. Some AYAs feel more comfortable with peer support and this should be encouraged. Lastly, it is very important that the AYA is completely informed about their own medical and surgical history (Table 4.4) [96]. Gaining confidence in the new doctor and building a new trusting therapeutic relationship is important for successful transition and may require several combined visits with the pediatric and adult specialist. Many specialists for adult care are very unfamiliar with DSD and

Table 4.4 Adapted checklist for assessing if an adolescent or young adult with DSD is prepared to transition their healthcare to adult medical specialists.

Healthcare skills	Health history knowledge
I have decided who will be my DSD physician for adult care	What is DSD? What type of DSD do you have?
I can explain my DSD to unfamiliar physicians	What medications do you currently take? How much and how often?
I can find information about my condition online, and I know how to connect with DSD advocacy and support groups	Why do you take these medications? What happens if you take too much or too little?
I schedule and keep a calendar of my own medical appointments	When and how was your DSD diagnosed?
I wear medic alert jewellery to alert others of my life-threatening allergies or conditions (when needed)	Have you had any surgeries for your DSD? If yes, what procedures and when?
I prepare and ask questions to my healthcare team members	Do you have a copy of your medical records? If not, do you know how to get this?
I can explain side effects of my medications	What is the natural history of your DSD (if known)?
I can explain complications associated with my DSD and how to avoid them	Are you fertile?
I know what symptoms of my DSD require urgent care and where to go for that care	Do you know what kinds of mental health and healthcare specialists are available to help you as you get older (e.g. psychiatrist, reproductive endocrinologist, couples counsellor)?
I can describe how my DSD affects pubertal development, sexual functioning and fertility	
I keep records of my menstrual periods (when needed)	
I am informed about family planning and have access to contraceptives (when needed)	
I know how to connect with genetic counsellors to discuss my condition	
I perform breast self-exams (when needed)	
I perform testicular self-exams (when needed)	

Source: Reprinted from [94] with permission.

the pediatric multidisciplinary DSD team has an important role in educating adult specialists and local healthcare providers [97].

Adult Outcome

Several outcome studies of individuals with DSD have been performed. Most included large cohorts of patients with CAH but only a few or heterogeneous groups of patients with rare XY conditions, such as partial and complete gonadal dysgenesis, 45,X/46,XY mosaicism and androgen synthesis or action defects, have been reported. Major outcome measures of these studies were quality of life, psychological well-being, general health and sexual functioning. Results are inconsistent, with some studies showing rather good and others poor overall QoL. Women with CAH generally reported better QoL than women with XY conditions [95, 96]. Most problems reported in adulthood are psychological distress, impaired body image and sexual function [7, 98, 99]. One recent German multicentre study, which included CAH and XY conditions, showed that individuals with CAH and younger patients are more satisfied with care. Participants of a single-centre Italian study, which was performed in collaboration with the local support group, also reported better psychosocial adaptation in younger patients [7, 100].

A study from Brazil showed normal QoL in patients treated in a multidisciplinary single centre. It was suggested that early multidisciplinary care including psychological support was paramount here [101]. These results indicate that improvement of care rather than the condition itself is a major factor predicting positive outcome in adults with DSD. Another factor contributing to

better outcomes may be societal changes, with more openness towards the bimodal continuum of sex and gender. None of the studies reported high prevalence of gender change but results should be interpreted cautiously since all studies are prone to selection bias because satisfied individuals are more likely to participate in such outcome studies than those who are less satisfied. Furthermore, existing QoL questionnaires may be not sufficiently specific to detect the impact of the DSD on psychological well-being.

Other Examples of XY DSD

A number of relatively common abnormalities of XY sex development come under the generic umbrella of DSD but do not present with ambiguous genitalia. These include hypospadias and cryptorchidism, the latter occasionally associated with persistent Müllerian duct syndrome (PMDS).

Hypospadias

Hypospadias, incomplete fusion of the penile urethra, is defined by an arrest in the development of the urethral spongiosum and ventral prepuce; it has a birth prevalence of 3.5–7 per 1000 live male births [102] with chordee in the more severe cases. A simple classification comprises glandular, penile (mid-shaft) and perineoscrotal hypospadias (the most severe form). Environmental and genetic factors associated with hypospadias have been identified, although the aetiology in most individual cases is unknown. There is an important association with fetal growth restriction; in addition, some overlap between mutations in genes causing hypospadias and DSD exist, such as mutations in *AR*, *NR5A1* and *WT1* [103].

Urogenital anomalies associated with apparently isolated hypospadias are sufficiently common to recommend screening of all asymptomatic hypospadias patients with urinary tract ultrasound, cystogram, urinalysis and urine culture [104]. There are few outcome data on hormonal function, fertility and sexual function in adults who had hypospadias during childhood but there is evidence of some disturbance in psychosocial functioning during adolescence, delayed initiation of sexual activity, difficulties with ejaculation and a higher incidence of abnormal spermatogenesis [105]. Given the overlap with DSD, boys with isolated mid-shaft or proximal hypospadias benefit from endocrine workup.

The surgical procedures required to relocate the urethral opening on to the glans and correct chordee, which enable satisfactory cosmetic and functional outcomes to be achieved, are evolving. A Pediatric Penile Perception Score has been devised based on the assessment of the meatus, glans, skin and general appearance as judged by patients, their parents and the urologist [106]. The method appears to be reliable in assessing penile self-perception in children after hypospadias repair, who generally report high satisfaction with penile appearance comparable to age-matched controls.

Cryptorchidism

Cryptorchidism, which includes some cases of anorchia, is the most common congenital anomaly in boys, affecting 2–9% male live births. Factors to consider in aetiology include a strong association with low birth weight, as well as associations with maternal smoking and alcohol use, gestational diabetes and possible exposure to environmental chemicals. A higher prevalence in hypogonadotropic hypogonadism and AIS affirms the role of androgens in testicular descent, particularly during the second inguinoscrotal phase. The initial transabdominal phase of testis descent is under the control of insulin-like factor 3 (INSL3) and its receptor (LGR8/GREAT), although mutations in these two genes have only rarely been implicated in the aetiology of cryptorchidism. It is apparent that an interrelationship between hormones, genes and the environment (critical fetal exposure to antiandrogenic and oestrogenic compounds) underlies the multifactorial aetiology of cryptorchidism [107].

Orchidopexy is generally the treatment of choice for an undescended testis that is clearly distinguishable from a retractile testis. The link between testicular cancer in adulthood and previous testicular maldescent is well established and there is unequivocal evidence that performing orchidopexy before puberty significantly decreases the risk of testicular cancer [108]. In order to preserve optimal chances for future fertility, it is recommended that orchidopexy be performed before the age of 1 year [109].

Persistent Müllerian Duct Syndrome

Maldescent of the testis in this syndrome is mechanical in nature because of its attachment to a fallopian tube and uterus in the male deficient in AMH or its effect as a result of either a mutation in the *AMH* gene or in the gene that encodes for the AMH type II receptor [110]. A mutation is identified in more than 80% of cases. Serum AMH is low or undetectable in cases with an *AMH* gene mutation, whereas it is in the normal range with an AMH type II receptor mutation. External male genital development is normal in this syndrome. The diagnosis may be

established at orchidopexy for an undescended testis or during an inguinal hernia repair when the sac is found to contain a uterus or fallopian tube. Sometimes the sac also contains the contralateral testis and tube. Such transverse testicular ectopia is, *de facto*, a diagnosis of PMDS.

Surgical management seeks to place the testis in the scrotum but there is a risk of compromising the blood supply and damaging the vas deferens and conservative treatment may be more appropriate in the case of transverse testicular ectopia when both testes are already sited ipsilaterally in the scrotum. There appears to be no contraindication to leaving the uterus *in situ*.

Future Perspectives

With more widespread availability of novel diagnostic tools, such as next-generation sequencing techniques and multisteroid analysis, a more precise molecular diagnosis will become possible in many cases. New genes and pathways are being discovered at a rapid pace. Increasing knowledge of pathogenetic mechanisms of gonadal development and gonadal histology in combination with genetic risk profiling and development of new serum tumour markers will lead to individualized decision-making regarding gonadal preservation. In many countries genital surgery is postponed until the patient can give informed consent but no data have been reported on the benefits and disadvantages of delaying reconstructive genital surgery and/or gonadectomy.

Children and teenagers are increasingly involved in decision-making, e.g. regarding start of hormone therapies. Disclosure of medical information in an age-appropriate and sensitive manner should be started in early childhood. Psychological counselling and sexual education are also needed. Psychological adjustment tools should be developed to evaluate the needs of patients and families.

Novel techniques of assisted reproduction are available in many countries and infertility treatment by oocyte or sperm donation or even uterus transplantation and cell reprogramming may become future options for some patients with complete gonadal dysgenesis or absent uterus. Initiatives to provide education for healthcare professionals through interaction with patient representatives and support groups should be developed to reduce stigmatization of individuals who have unexpected sexual characteristics. In addition, healthcare professionals are well placed to promote and accelerate the acceptance of non-normative societal concepts of sex and gender. Participation in and representation by persons living with a DSD in these ethical and societal issues should be further encouraged.

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Weblinks

- http://www.espe-elearning.org/: educational module supported by the European Society for Pediatric Endocrinology for professionals and trainees who wish to extend their theoretical and clinical knowledge on DSD as well as test their communication skills. This includes several interactive cases, training and self-assessment tools.
- http://www.erasmusmc.nl/pathologie/research/lepo/4530687/: animated tool describing normal and atypical sex development, with focus on gonadal development and the biology of germ cell tumours in DSD, supported by the Erasmus University Rotterdam.
- http://androgendb.mcgill.ca/: androgen receptor database, summarizing all scientific information on AR function and AR mutations, regularly updated.
- www.i-dsd.org: International DSD Registry, currently containing data on over 3000 individuals who have a DSD condition. Data can be inputted by medical professionals, provided that informed consent has been obtained. The I-DSD Registry is used as a clinical database and a research tool.
- www.dsdfamilies.org: an online information and support resource for families with children, teens and young adults who have a DSD. The website also offers links to other patient organizations worldwide.

5

Disorders of Hypothalamo-Pituitary Axis

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KEY POINTS

- The neuroendocrine network between the hypothalamus and pituitary gland controls growth, reproduction, metabolism, stress responses and salt and water homeostasis by regulating a wide range of peripheral target organs.
- The function of these pathways can be disrupted by congenital malformations or by acquired causes resulting in anatomical damage to the region, leading to either endocrine deficits or, more rarely, syndromes of hormonal excess.
- Many genes involved in the development of the hypothalamo-pituitary axis have been discovered with the identification of single-gene mutations causing hypopituitarism. Congenital hypopituitarism is almost always idiopathic and likely to involve multiple genes and/or environmental factors.
- Acquired hypopituitarism can result from a variety of insults to the central nervous system, including tumours, tumour-related treatments, inflammation, infiltration, infection, trauma, iron deposition and emotional abuse.
- Regardless of the cause, hypopituitarism has the potential to evolve over time, with deficits recurring and resolving; thus all patients require lifelong follow-up with clear plans for transition to adult services.

Introduction

The neuroendocrine network between the hypothalamus and pituitary gland is responsible for the regulation of growth, reproduction, metabolism and homeostasis by coordinating signals from the brain to various target organs such as the adrenals, thyroid and gonads. The network of neurovascular and endocrine structures lies deep within the brain parenchyma, with the pituitary gland in a bony cavity known as the sella turcica (Figure 5.1). The hypothalamus lies superior to this, surrounding the third ventricle and formed by a dense conglomeration of nuclei that secrete stimulatory and inhibitory releasing hormones into the hypothalamo-hypophyseal portal vascular system (derived from the superior hypophyseal arteries and draining into the vein of Galen) that regulate anterior pituitary cells or project axons (originating from the magnocellular neurons of the paraventricular and supraoptic hypothalamic nuclei) into the posterior pituitary gland from where oxytocin (OXT) and arginine vasopressin (AVP) are secreted into the circulation. The pituitary stalk, or infundibulum, carries both the portal vasculature to the anterior pituitary and the neural tracts to the posterior pituitary gland, so that damage to it can easily result in panhypopituitarism.

The prevalence of hypopituitarism in the general population is estimated to be between 290 and 455 per million, with an annual incidence rate of 42.1 cases per million [1]. The proximity of this network to surrounding structures including the optic chiasm, third ventricle, cavernous sinuses, thalamus and basal ganglia means that congenital and acquired disorders involving this region often result in hypothalamo-pituitary dysfunction. This chapter covers the common causes, clinical features, diagnostic evaluation and management of hypothalamo-pituitary disease; the effects of surgery and radiotherapy for central nervous system (CNS) tumours are covered in Chapter 13 ('Endocrine Late Effects of Cancer Treatment').

Brook's Clinical Pediatric Endocrinology, Seventh Edition. Edited by Mehul T. Dattani and Charles G. D. Brook.

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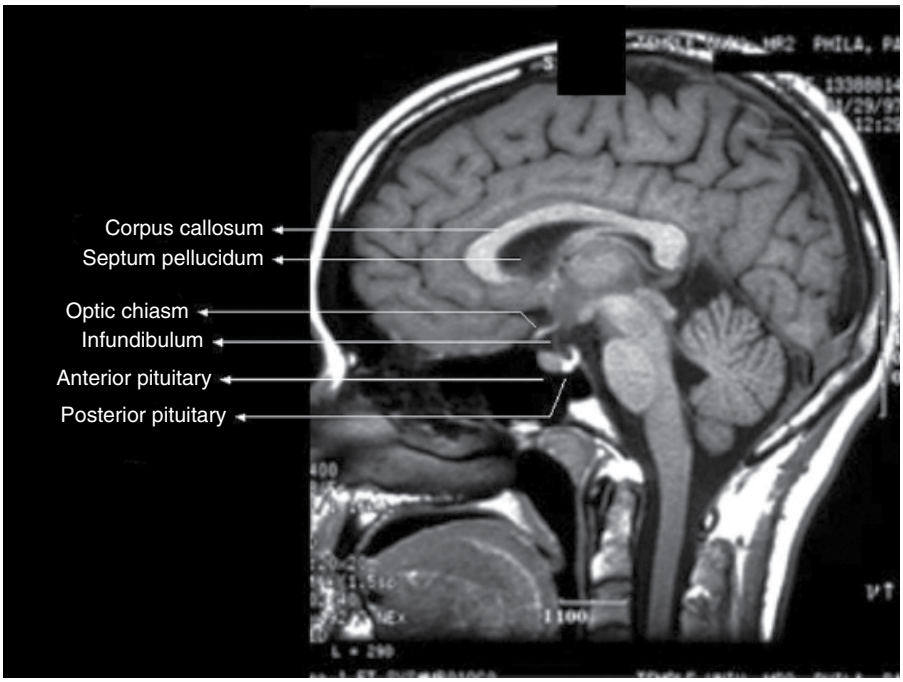

Figure 5.1 T_1-weighted sagittal MRI image illustrating the anatomical relationships of the pituitary gland. The hypothalamus (not labelled here) is the region directly above the infundibulum.

The Hypothalamo-Pituitary Neuroendocrine Axis

The hypothalamus consists of a dense congregation of nuclei lying superior to the pituitary gland, surrounding the third ventricle. It has numerous axonal projections to the cerebral cortex, brainstem, reticular formation, limbic system and autonomic nervous system, many circuits of which are only beginning to be understood. The hypothalamus regulates the anterior pituitary gland by secreting stimulatory and inhibitory releasing peptide hormones into a capillary plexus in the median eminence, whence they are carried in the bloodstream via the hypothalamo-hypophyseal portal vascular system to the pituitary. Magnocellular neurons from the paraventricular and supraoptic nuclei of the hypothalamus have direct axonal projections to the posterior pituitary (the hypothalamo-neurohypophyseal tract) and are responsible for synthesizing OXT and AVP (also known as antidiuretic hormone [ADH]) (Table 5.1, Figure 5.2). Both the portal system and axonal projections are carried to the pituitary gland by the infundibulum (pituitary stalk), which therefore forms a critical structural connection.

The mature pituitary gland consists of the adenohypophysis (anterior and intermediate lobes) and neurohypophysis (posterior lobe). The adenohypophysis consists of six different cell types, each described by the hormone it produces and by the nature of their appearances on haematoxylin and eosin (H&E) staining: the acidophils are somatotrophs (growth hormone [GH]) and lactotrophs (prolactin [PRL]); the basophils are gonadotrophs (luteinizing hormone [LH] and follicle-stimulating hormone [FSH]), corticotrophs (adrenocorticotropic hormone [ACTH]) and thyrotrophs (thyroid-stimulating hormone [TSH]); the chromophobes are melanotrophs (α-melanocyte-stimulating hormone [α-MSH] and β-endorphins, both breakdown products of pro-opiomelanocortin [POMC]). By adulthood, the intermediate lobe, where melanotrophs are largely located, has involuted and is avascular [2]. The neurohypophysis contains the termini of hypothalamic axonal projections that secrete AVP and OXT directly into the bloodstream via the surrounding capillary network. The relationship between the various pituitary hormones, their hypothalamic stimulating or inhibitory counterparts and the end organs on which they act is summarized in Figure 5.2.

Hypothalamo-Pituitary Development

Although there is little direct evidence for pituitary development in humans, the process is highly conserved across all vertebrates [3] and development of the pituitary gland in mouse models is well characterized (Figure 5.3). The pituitary gland has a dual embryonic

Table 5.1 Hormone secreted by the anterior and posterior lobes of the pituitary gland and their relationships to the various hypothalamic regulatory hormones that are in turn secreted by various hypothalamic nuclei.

Pituitary hormone	Cell type	%Anterior pituitary cell population	Hypothalamic hormone(s)	Peptide length	Hypothalamic nuclei
GH	Somatotroph	40–50%	GHRH (+) SS (–)	44 a.a's 14 a.a's	Arcuate, paraventricular
LH/FSH	Gonadotroph	10–15%	GnRH (+)	10 a.a's	Medial preoptic, arcuate, paraventricular
ACTH	Corticotroph	15–20%	CRH (+) AVP (augments CRH)	41 a.a's	Paraventricular, arcuate
TSH	Thyrotroph	3–5%	TRH (+) SS (–)	3 a.a's 14 a.a's	Paraventricular, arcuate
PRL	Lactotroph	10–25%	TRH (+) DA (–)	3 a.a's 1 a.a	Paraventricular Arcuate
AVP	Hypothalamic magnocellular neurons	—	—	9 a.a's	Paraventricular, supraoptic
OXT	Hypothalamic magnocellular neurons	—	—	9 a.a's	Paraventricular, supraoptic

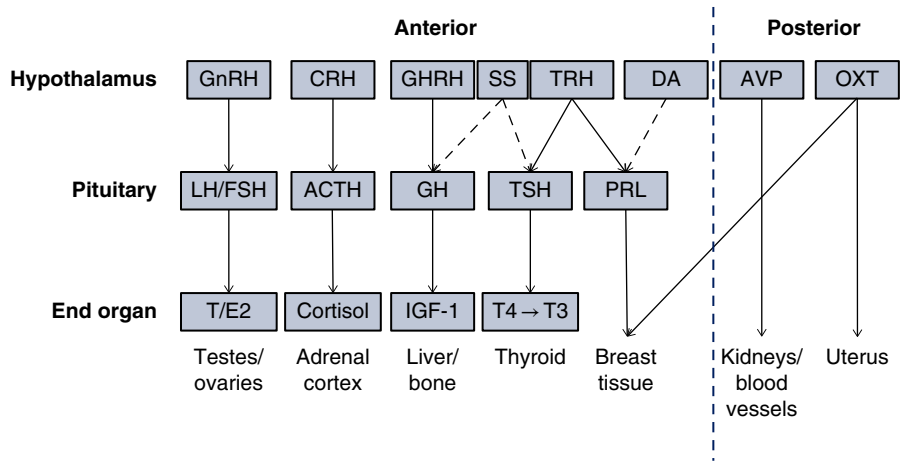

Figure 5.2 Relationship between pituitary hormones, their hypothalamic stimulating (solid arrows) and inhibitory (broken arrows) counterparts, and the end organs they act on. GnRH, gonadotropin-releasing hormone; CRH, corticotropin-releasing hormone; GHRH, growth hormone-releasing hormone; SS, somatostatin; TRH, thyrotropin-releasing hormone; DA, dopamine; AVP, arginine vasopressin (antidiuretic hormone); OXT, oxytocin; LH, luteinizing hormone; FSH, follicle-stimulating hormone; GH, growth hormone; TSH, thyroid-stimulating hormone; PRL, prolactin; T, testosterone; E2, oestradiol; IGF-1, insulin-like growth factor 1; T4, thyroxine; T3, triiodothyronine.

origin: the anterior and intermediate lobes are derived from oral ectoderm, the posterior pituitary from neural ectoderm [5–8]. The close apposition and interaction of these two ectodermal layers throughout neurodevelopment is crucial to the formation and function of a normal pituitary gland [8, 9] and is closely linked to the development of the hypothalamus. This process is dependent upon the sequential temporal and spatial expression of a cascade of signalling molecules and transcription factors that play a crucial role in organ commitment, cell proliferation, patterning and terminal differentiation.

Hypothalamo-Pituitary Organogenesis

The anterior pituitary develops from the hypophyseal or pituitary placode, one of the six cranial placodes that appear transiently as localized ectodermal thickenings in the prospective head of the developing embryo. The pituitary placode appears at mouse embryonic day (E) 7.5, located ventrally in the midline of the anterior neural ridge in continuity with the future hypothalamo-infundibular region located posteriorly in the rostral part of the neural plate [10, 11]. By E8.5, the neural tube bends at the cephalic end and the pituitary placode is now a

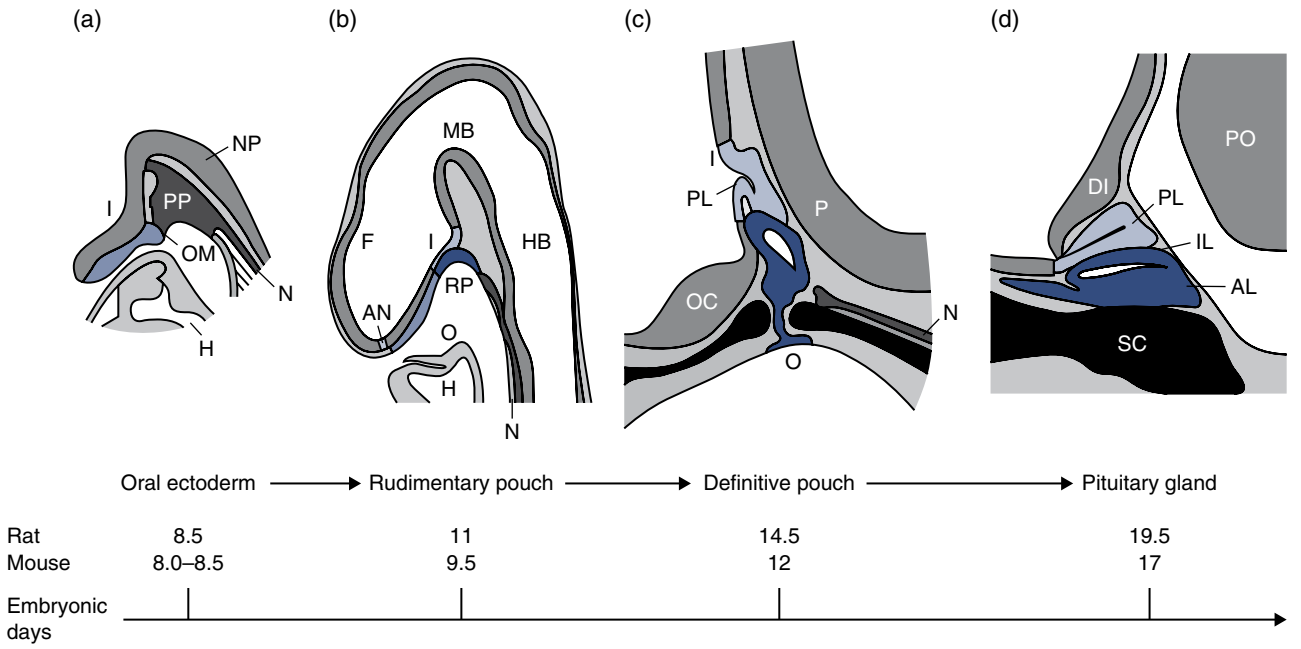

Figure 5.3 Rodent pituitary development indicating the four main stages by days' post-conception (dpc). Development commences with (a) thickening of the stomodeum (oral ectoderm) at 8.5 dpc, followed by (b) invagination of the rudimentary Rathke's pouch (RP) to keep it in contact with the overlying ventral diencephalic neuroectoderm. (c) The RP is definitively formed when the connection with the oral ectoderm is severed; concurrently the ventral diencephalon invaginates to form the infundibulum (I) and posterior pituitary. (d) Progenitors of the hormone-secreting cell types proliferate and differentiate to form the mature pituitary gland consisting of anterior (AL), intermediate (IL) and posterior lobes (PL). NP, neural plate; N, notochord; PP, pituitary placode; OM, oral membrane; H, heart; F, forebrain; MB, midbrain; HB, hindbrain; AN, anterior neural pore; O, oral cavity; OC, optic chiasm; P, pontine flexure; DI, diencephalon; SC, sphenoid cartilage. *Source:* Taken from Sheng and Westphal [4]).

thickening of the roof of the primitive oral cavity [8, 9]. The onset of pituitary organogenesis corresponds to 4–6 weeks of gestation in humans.

At E9.0, the placode invaginates and forms the rudimentary Rathke's pouch from which the anterior and intermediate lobes of the adenohypophysis are derived [12, 13]. The pouch eventually folds on itself and begins to close off by E10.5, while the neural ectoderm at the base of the developing diencephalon evaginates to give rise to the posterior pituitary [14]. Between E10.5 and 12.0, the pouch epithelium continues to proliferate and is completely detached from the oral cavity at E12.5 to form the definitive Rathke's pouch. The progenitors of the hormone-secreting cell types proliferate ventrally from the pouch between E12.5 and 17.5 and populate what will form the mature anterior lobe [15, 16]. The remnants of the dorsal portion of the pouch will form the intermediate lobe, while the lumen of the pouch remains as the pituitary cleft separating the two lobes of the adenohypophysis.

Concomitantly with these events, the hypothalamic primordium becomes morphologically evident in the neural ectoderm at E9.5 with hypothalamic neurogenesis commencing at E10, concurrent with the highest level of expression of genes important for the regional patterning of hypothalamic progenitor cells, such as *Sim1*, *Sim2*, *Arx* and *Nr5a1* [17]. Hypothalamic neurogenesis is complete by E16 although expression of hypothalamic terminal differentiation markers peaks postnatally [17].

Cell Differentiation, Organization and Plasticity in the Anterior Pituitary

The differentiated hormone-producing cells of the developing pituitary gland arise in a temporally and spatially regulated manner. The earliest marker of differentiation in the anterior pituitary is the expression of α-glycoprotein subunit (αGSU) (encoded by *Cga*) by E11.5 in a restricted patch of cells in the ventral Rathke's pouch [9]. These cells correspond to prospective thyrotrophs that also express the transcription factor islet-1 (*Isl1*) and will differentiate at E12.5 by initiating the expression of thyroid-stimulating hormone β-subunit (*Tshβ*) [9]. This early cell population is transient and disappears at birth; in the mature adult pituitary, αGSU is detected only in definitive thyrotrophs and gonadotrophs [18–20].

Corticotrophs, defined by the expression of *Pomc*, start to differentiate at E12.5 in the area dorsal to the prospective thyrotrophs [18, 19, 21]. Definitive thyrotrophs, characterized by *Tshβ* expression, are detected

by E14.5. The expression of *Gh* (growth hormone) and *Prl* (prolactin) by E15.5 is the hallmark of differentiation of the somatotroph and lactotroph lineages, respectively, with the latter appearing in the anterolateral wings of the developing gland following which they increase dramatically in number and migrate by E18.5 throughout the central and lateral parts of the anterior lobe. The lactotrophs remain localized to the medial zone adjacent to the ventral surface of the intermediate lobe. Gonadotrophs are the last cell lineage to appear, beginning at E16.5 with the onset of expression of the β-subunit of LH (*Lhβ*) and subsequently FSH (*Fshβ*) a day after [9, 19].

The classic description of sequential cell differentiation has recently been challenged by birth-dating studies that imply the earlier specification of endocrine cells that migrate to their designated locations, suggesting a broader range of specification rather than a sequential pattern of discrete timings [22]. The organization of hormone-producing cells within the pituitary is not random: cells maintain structural and functional homotypic networks facilitating a coordinated physiological response to stimuli contributing to the plasticity of the gland [23–25]. For instance, the network organization of lactotrophs increases their functional connectivity and communication resulting in increased hormonal output and also allowing the development of plasticity and 'memory', whereby augmented output of hormones is maintained during a second lactation in subsequent pregnancies.

The identification of *Sox2*-positive pituitary progenitor (stem) cells in the developing and adult pituitary in the area lining the pituitary cleft has additionally changed our understanding of pituitary development. These cells maintain their ability to self-proliferate, forming pituispheres *in vitro*, and can be induced to differentiate into any pituitary lineage, as demonstrated by the generation of a functional three-dimensional anterior pituitary gland from mouse embryonic stem cells placed in diverse culture conditions and exposed to various induction factors [26]. They generate hormone-producing cells during embryonic development as well as in adulthood and therefore contribute to pituitary homeostasis, are implicated in the regenerative potential of the pituitary and are able also to act in a non-cell-autonomous manner to induce oncogenesis.

Early Developmental Genes and Transcription Factors

The development of the anterior pituitary gland is dependent upon a cascade encoding extrinsic and intrinsic transcription factors and signalling molecules that are expressed and silenced in a temporally and spatially restricted manner. Extrinsic molecules from the ventral diencephalon (BMP4, FGF8, FGF4, NKX2-1, WNT5A), oral ectoderm (sonic hedgehog [SHH]), surrounding mesenchyme (BMP2, Indian hedgehog [IHH], chordin) and the pouch itself (BMP2, WNT4) create a network of signalling gradients important for early pituitary morphogenesis (Figure 5.4).

Spontaneous and artificially induced mutations in the mouse have led to significant insights into human pituitary disease. Identification of mutations associated with human pituitary disease has also been invaluable in defining the genetic cascade of pituitary development. The advent of new technologies such as whole exome and whole genome sequencing has resulted in an expanding list of genetic factors involved in human hypothalamo-pituitary disease. Examples of genetic factors involved in isolated or multiple pituitary hormone deficiencies are listed in Tables 5.2 and 5.3, respectively. Their phenotypes will be discussed in the next section.

Morphogenetic Signals (BMPs, FGFs, SHH, Wnt/β-Catenin Pathway)

The initial induction and maintenance of Rathke's pouch depends on the expression of bone morphogenetic protein 4 (BMP4) and fibroblast growth factor 8 (FGF8). BMP4 is the earliest secreted molecule detected in the prospective infundibulum from E8.5 to E14.5 [18, 82]. Following the onset of *Bmp4* expression, FGF8 and other members of the FGF family (*Fgf1*, *Fgf18*) are expressed in the diencephalon from E9.5 [8, 18, 64, 83, 84]. FGF signalling is necessary for cell proliferation within the pouch and the activation of LIM homeobox 3 (*Lhx3*) and LIM homeobox 4 (*Lhx4*) is essential for the development of the definitive pouch from the rudimentary pouch [6, 18, 85–88].

SHH and its downstream signalling pathway of activators (GLI2) or repressors (GLI3) are also important for the patterning of the gland and specification and expansion of ventral cell types. *Shh* is expressed in the ventral diencephalon and the oral ectoderm with maintenance in the former until E14.9; its expression is excluded from Rathke's pouch as soon as the pouch appears [83, 84]. A number of Wnt family members (WNT4, WNT5A) and components of the downstream signalling pathway (β-catenin) are expressed within Rathke's pouch, in diencephalon and in the surrounding tissues [84, 89, 90]. Members of the Wnt/β-catenin signalling pathway interact with other early transcription factors (SOX2 [91], SOX3 [92]) and these interactions are important for various developmental processes, including that of the pituitary gland.

Early Transcription Factors

Hesx1

Hesx1 is a member of the paired-like class of homeobox genes and one of the earliest markers of the pituitary

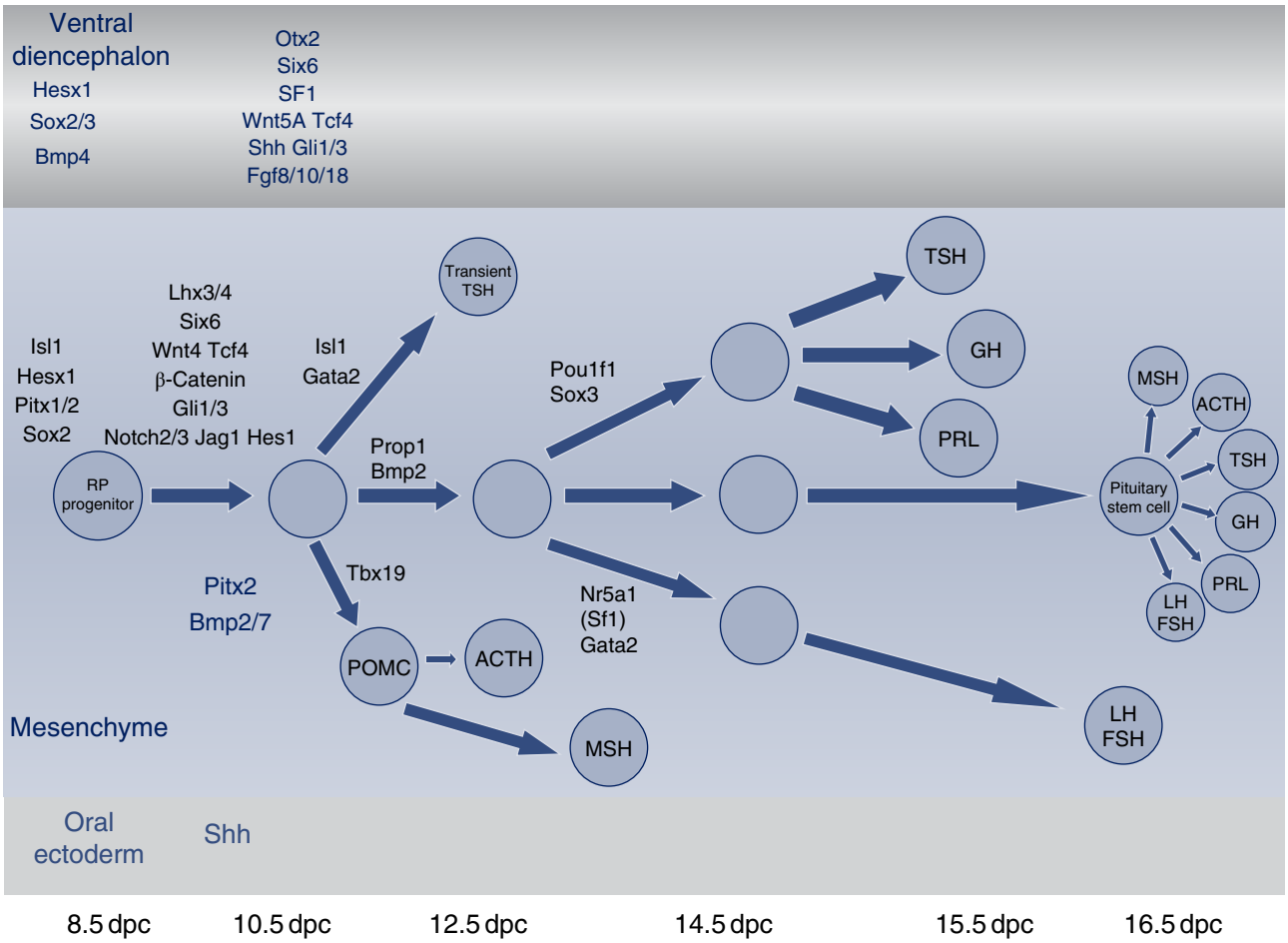

Figure 5.4 Schematic representation of the developmental cascade of transcription factors and signalling molecules involved in anterior pituitary development. *Source:* Taken from Kelberman et al. [9]. Reproduced with permission of Oxford University Press.

primordium [93]. From E9.0 to 9.5, its expression is restricted to the ventral diencephalon and the developing Rathke's pouch, subsequently gradually disappearing in a spatio-temporal sequence corresponding to progressive pituitary cell differentiation and becoming undetectable by E15.5 [6, 30, 94–96]. *Hesx1* is a transcriptional repressor and its downregulation is important for downstream activation of *Prop1* and the emergence of the *Pou1f1* cell lineage (somatotrophs, thyrotrophs and lactotrophs) [6]. The concomitant repression of *Hesx1* and activation of *Pou1f1* is mediated by the direct interaction between β-catenin and PROP1 [97]. *Hesx1*-null mice ($Hesx1^{-/-}$) have a variable phenotype reminiscent of patients with septo-optic dysplasia (SOD) with absent optic vesicles, severe microphthalmia, hypothalamic abnormalities and abnormal morphogenesis of Rathke's pouch (Table 5.4) [30]. The pituitary may be more sensitive to changes in *Hesx1* dosage than the eyes or the forebrain [98].

LIM Family of Homeobox Genes (Lhx3, Lhx4, Lhx2)

Lhx3 is one of the earliest transcription factors expressed in Rathke's pouch (E9.5) and in the developing anterior and intermediate pituitary (E16.5) but not in the posterior lobe; its expression persists into adulthood [99, 100]. *Lhx3* is important for the establishment of hormone-producing cell types and the maintenance of at least some cell types in the mature pituitary [101, 102]. *Lhx4* is also expressed in Rathke's pouch and is downregulated by E15.5 [99]. *Lhx4* is additionally expressed in areas of the developing hindbrain, cerebral cortex and motor neurons of the spinal cord [101, 103]. The phenotypes of *Lhx3*- and *Lhx4*-null mice are shown in Table 5.4. Double mutants ($Lhx3^{-/-}$ $Lhx4^{-/-}$) have a more severe phenotype than either single mutant, suggesting that there is redundancy in their actions [4]. More recently *Lhx2* was shown to be expressed in the diencephalon and posterior lobe [104].

SOX family of transcription factors (Sox2 *and* Sox3)

The SOX family of transcription factors is characterized by the presence of a 79-amino-acid high mobility group (HMG) DNA-binding domain similar to the HMG domain of the mammalian sex-determining gene *SRY*. *Sox2* and *Sox3* are expressed throughout the developing

Table 5.2 Genetic disorders of hypothalamo-pituitary development resulting in isolated pituitary deficiencies.

Gene	Phenotype	Inheritance
GH1	GH deficiency	AR, AD
GHRHR	GH deficiency	AR
RNPC3	GH deficiency	AR
KAL1	Kallmann syndrome, unilateral renal agenesis, synkinesis	XL
FGFR1	Kallmann syndrome, normosmic hypogonadotropic hypogonadism, variable gonadotropin deficiency, cleft lip/palate, abnormalities of corpus callosum	AD
FGF8	Kallmann syndrome, normosmic hypogonadotropic hypogonadism, variable gonadotropin deficiency, cleft lip/palate, camptodactyly	AD
PROK2	Kallmann syndrome, obesity	AD, AR
PROKR2	Kallmann syndrome	AD, AR
HS6ST1	Kallmann syndrome	AD
WDR11	Kallmann syndrome	AD
SEMA3A	Kallmann syndrome	AD
SOX10	Kallmann syndrome, Waardenburg syndrome (deafness, skin/hair/iris hypopigmentation)	AD
FEZF1	Kallmann syndrome	AR
GNRH1	Normosmic hypogonadotropic hypogonadism	AR
GNRHR	Normosmic hypogonadotropic hypogonadism	AR
KISS1 (kisspeptin)	Normosmic hypogonadotropic hypogonadism	AR
KISS1R (formerly *GPR54*, kisspeptin receptor)	Normosmic hypogonadotropic hypogonadism	AR
LEP (leptin)	Normosmic hypogonadotropic hypogonadism, obesity	AR
LEPR (leptin receptor)	Normosmic hypogonadotropic hypogonadism, obesity	AR
TAC3	Hypogonadotropic hypogonadism	AR
TAC3R	Hypogonadotropic hypogonadism	AR
CHD7	Hypogonadotropic hypogonadism, Kallmann syndrome, CHARGE variants	AD
FSHβ	Primary amenorrhoea, defective spermatogenesis, low FSH	AR
LHβ	Delayed puberty, low or elevated LH	AR
DAX1	Hypogonadotropic hypogonadism, congenital adrenal hypoplasia	XL
TSHβ	TSH deficiency	AR
TRHR	TSH deficiency	AR
IGSF1	TSH ± PRL deficiencies, transient GH deficiency, macroorchidism (males), ovarian cysts (females)	XL
PC1	ACTH deficiency, hypoglycaemia, hypogonadotropic hypogonadism, obesity	AR
POMC	ACTH deficiency, obesity, red hair	AR
CRH	CRH deficiency	AR
AVP-NPH	Central diabetes insipidus	AR, AD

AR, autosomal recessive; AD, autosomal dominant; XL, X-linked.

CNS and are some of the earliest markers in neuronal determination. During pituitary development *Sox3* is expressed in the ventral diencephalon and infundibulum but not in Rathke's pouch [105, 106]. Deletion of *Sox3* in mice results in a complex and variable phenotype: one-third have no gross abnormalities but the more severely affected exhibit profound GH insufficiency, reduction in size, infertility, hypopituitarism and midline and craniofacial defects. The endocrine deficits are variable with pituitary levels of GH, LH, FSH and TSH being lower than in wild-type animals. The embryonic pituitaries of *Sox3* mutants have an abnormally expanded and bifurcated Rathke's pouch (E11.5), which possibly results in the additional cleft observed in adult pituitaries. The evagination of the infundibulum is less pronounced and the presumptive hypothalamus is

Table 5.3 Genetic disorders of hypothalamo-pituitary development resulting in syndromic hypopituitarism or combined pituitary hormone deficiencies.

Gene	Endocrine phenotype	MRI phenotype	Other associations	Inheritance
Syndromic hypopituitarism				
HESX1	Panhypopituitarism; GH deficiency; GH with evolving ACTH and TSH deficiencies	APH, EPP, ONH, ACC; normal AP with EPP and ONH (p.S170L)	Septo-optic dysplasia and its variants	AD [27–29]
	Panhypopituitarism; GH, LH, FSH, evolving ACTH and TSH deficiencies	APH, EPP, ONH, ACC; normal ON with EPP and APH (p.I26T); pituitary aplasia with normal PP and ON; pituitary aplasia with normal PP and ON coloboma		AR [30–33]
TCF7L1	Isolated GH deficiency or low IGF-1, mildly elevated TSH with normal fT_4	APH, absent PP, ONH, thin optic tracts, partial agenesis of the corpus callosum, absent septum pellucidum, thin anterior commissure	Septo-optic dysplasia	AD with variable penetrance [34]
LHX3	GH, TSH, PRL, LH, FSH deficiencies; ACTH deficiency	APH, enlarged/cystic AP, normal PP and stalk	Limited neck rotation, short cervical spine, sensorineural deafness	AR [35–38]
LHX4	Panhypopituitarism; GH with variable TSH, ACTH, LH and FSH deficiencies	APH, normal PP or EPP, Chiari malformation, cerebellar abnormalities	—	AD [39–41]
	ACTH, TSH, PRL and probable GH deficiencies	Pituitary aplasia, EPP	Lethality in the first weeks of life with severe sepsis, poor tone, lung atelectasis, midfacial hypoplasia, low-set ears	AR [42]
SOX2	LH and FSH deficiencies, rarely GH deficiency	APH, thin CC; hippocampal abnormalities; hypothalamic hamartoma; slow-progressing hypothalamo-pituitary tumour	Bilateral/unilateral anophthalmia, spastic diplegia, developmental delay, trachea-oesophageal fistula, sensorineural deafness	AD [43–47]
SOX3	Panhypopituitarism; GH, TSH, ACTH, LH and FSH deficiencies; isolated GH deficiency	APH, EPP; persistent craniopharyngeal canal	Variable mental retardation	XL [48–52]
OTX2	Isolated or partial GH deficiency; GH, TSH, ACTH, LH and FSH deficiencies	Normal pituitary; APH, EPP, Chiari malformation	Anophthalmia, bilateral/unilateral retinal dystrophy; normal eye phenotype	AD [53–56]
ARNT2	Central diabetes insipidus, GH, ACTH, TSH deficiencies	APH, absent PP, thin CC; frontal and temporal lobe hypoplasia, large Sylvian fissure	Microcephaly, seizures, visual impairment, renal tract abnormalities	AR [57]
GLI2	Panhypopituitarism; GH, TSH, ACTH, LH and FSH deficiencies; isolated GH deficiency	APH, normal PP or EPP, hypoplastic PP, ONH, holoprosencephaly, cavum septum pellucidum	Midfacial defects, cleft lip/palate, single central incisor, postaxial polydactyly	AD [58–61]
PITX2	Reduced GH concentration	Sella turcica hypoplasia	Axenfeld-Rieger syndrome (malformation of the anterior segment of the eye, dental hypoplasia, protuberant umbilicus, brain abnormalities)	AD [62, 63]
FGF8[a]	Borderline peak GH concentration	ACC, ONH	Moebius syndrome, spastic diplegia, developmental delay	AD [64, 65]
	Central diabetes insipidus, ACTH and TSH deficiencies	Semilobar holoprosencephaly, bulky AP, normal PP	Microcephaly, micrognathia, high arched palate	AR [64]

FGFR1[a]	GH, ACTH, TSH, LH and FSH deficiencies, central diabetes insipidus	APH, EPP, thin or normal pituitary stalk, ACC, ONH	Cleft lip/palate, brachydactyly, single central incisor	AD [65, 66]
PROKR2[a]	GH, ACTH, TSH, LH and FSH deficiencies; isolated GH deficiency	APH or normal AP, EPP, absent pituitary stalk, CC dysgenesis	Facial asymmetry, schizencephaly, cerebellar hypoplasia, hypoplastic optic discs	AD [65–67]
PNPLA6	GH, TSH, LH and FSH deficiencies; isolated hypogonadotropic hypogonadism	APH, cerebellar hypoplasia	Oliver–McFarlane and Gordon-Holmes syndromes; ataxia, chorioretinal atrophy, spasticity	AR [68, 69]
KAL1[a]	GH, ACTH and TSH deficiencies	APH, ONH	Septo-optic dysplasia, females affected	XL [70]
Combined pituitary hormone deficiencies				
POU1F1	GH, PRL and TSH deficiencies (TSH deficiency may present early or develop later)	APH or normal AP, normal PP and infundibulum, no extra-pituitary abnormalities	—	AD, AR [71–75]
PROP1	GH, TSH, PRL, LH, FSH and evolving ACTH deficiencies with variable time of onset and severity	APH, normal or enlarged AP that may change over time, normal PP and stalk	—	AR [76–81]

ACC, absent corpus callosum; AD, autosomal dominant; AP, anterior pituitary; APH, anterior pituitary hypoplasia; AR, autosomal recessive; CC, corpus callosum; EPP, ectopic posterior pituitary; ON, optic nerves; ONH, optic nerve hypoplasia; PP, posterior pituitary; XL, X linked.
[a] These genes are also known to cause isolated pituitary hormone deficiencies.

Table 5.4 Pituitary phenotypes in murine models with spontaneous or induced disruption of expression of pituitary transcription factors and signalling molecules.

Gene	Murine model	Pituitary morphology	Other phenotypes
Bmp4	*Bmp4* KO	No pituitary placode or pouch formation	Early embryonic lethality
Fgf8	*Fgf8* KO		Early embryonic lethality
	Fgf8 hypomorph	Variable: normal to severe APH, with/without absent PP, midline defects indicative of holoprosencephaly	Reduced numbers of hypothalamic neurons secreting AVP and OXT
Shh	$Shh^{\Delta hyp}$ conditional deletion in hypothalamus	Abnormal invagination of Rathke's pouch, abnormalities of infundibulum	Reduced numbers of somatotrophs, corticotrophs and thyrotrophs, defective optic disc development
Wnt4	$Wnt4^{-/-}$	Pituitary hypoplasia	Reduced numbers of somatotrophs and thyrotrophs
Wnt5	$Wnt5a^{-/-}$	Abnormal and bifurcated pouch	
Hesx1	$Hesx1^{-/-}$	Abnormal morphogenesis of Rathke's pouch, pituitary aplasia, multiple invaginations and abnormal branching, apparent formation of multiple pituitary glands	Markedly decreased head size, reduction in the prospective forebrain, severe microphthalmia, absence of optic vesicles, optic cups and olfactory placodes, hypothalamic abnormalities
Tcf7l1	$Tcf7l1^{fl/-}$ conditional knockout in hypothalamus	Abnormal pituitary clefting with hyperplasia but normal differentiation, ectopic pituitary tissue in oropharynx with indistinguishable posterior/intermediate lobes	Microphthalmia, anophthalmia, small/absent telencephalic vesicles, neural tube defects (e.g. exencephaly), dwarfism
Lhx3	$Lhx3^{-/-}$	Arrested pituitary development	Early lethality
Lhx4	$Lhx4^{-/-}$	Anterior pituitary hypoplasia	Markedly reduced anterior pituitary cell lines
Lhx2	$Lhx2^{-/-}$	Failure of evagination of the neuroectoderm, disorganized anterior and intermediate lobes	Presence of all cell lineages
Sox3	Females: $Sox3^{-/X}$	Normal	Mild craniofacial phenotype
	Males: $Sox3^{-/-}$	Expanded and bifurcated Rathke's pouch, thinner presumptive hypothalamus, small AP, presence of additional abnormal cleft, dysgenesis of corpus callosum	40% do not survive to weaning, reduction in size and fertility, hypopituitarism, midline and craniofacial defects, overgrowth and misalignment of front teeth, abnormal or absent pinna
Sox2	$Sox2^{\beta geo/+}$ heterozygous loss of Sox2	Bifurcated pouch, APH, extra clefts in adult pituitaries	Reduced numbers of somatotrophs and gonadotrophs, reduced GH, LH, ACTH and TSH content
	Selective loss of *Sox2* from the pituitary	APH	Perinatal lethality, reduced differentiation of somatotrophs and thyrotrophs, reduced numbers of gonadotrophs and hypothalamic GnRH neurons
Prop1	Ames mouse	APH (reduction in size by almost 50%), abnormal looping	Severe proportional dwarfism, infertility, GH, TSH and PRL deficiencies, low plasma LH and FSH
Pou1f1	Snell mouse	APH	Absence of somatotrophs, lactotrophs and thyrotrophs
	Jackson mouse		

APH, anterior pituitary hypoplasia; KO, knockout; PPl, posterior pituitary.

thinner and shorter with a marked reduction in cell proliferation [105, 107, 108].

Sox2 is expressed from the earliest stages of murine development throughout the developing CNS as well as in sensory placodes, inner ear, cochlea and the developing lens, retina and optic nerve. By E11.5 *Sox2* is expressed uniformly in Rathke's pouch and the infundibulum and its expression persists in a small population of cells of the adult murine pituitary lining the pituitary cleft. These cells maintain their potential to proliferate and differentiate into all pituitary cell types, representing a progenitor/stem cell pool [26]. $Sox2^{-/-}$ null embryos die shortly after implantation [109] but heterozygous animals ($Sox2^{\beta geo/+}$) exhibit abnormal morphogenesis of the gland with a bifurcated pouch and subsequent extra clefts in some adult pituitaries. Embryonic pituitaries are

smaller with significantly reduced numbers of somatotrophs and gonadotrophs and reduced GH, LH, ACTH and TSH content [43].

The effects of the reduction of *Sox2* expression seem to be dose dependent since reduction of levels below 40% of normal results in anophthalmia in the affected mutants. Transgenic animals with selective absence of SOX2 in the developing pituitary gland (*Hesx1*$^{Cre/+}$, *Sox2*$^{fl/fl}$) have severe anterior pituitary hypoplasia detectable from E12.5 with a marked reduction in the expression of *Pou1f1* resulting in reduced differentiation of somatotrophs and thyrotrophs. Gonadotrophs remain present, although in smaller numbers compared with wild-type pituitaries, accompanied by a significant reduction of GnRH neurons suggesting that the hypogonadal phenotype may be due to hypothalamic dysregulation [110].

Transcription Factor 7-like 1 (Tcf7l1)

Members of the T-cell factor/lymphoid enhancer factor (TCF/LEF) family are major targets for interaction with β-catenin and contain a β-catenin-interacting domain at their N-terminus. Transgenic conditional knockout mouse embryos with selective absence of TCF7L1 (*Hesx1*$^{Cre/+}$, *Tcf7l1*$^{fl/-}$), a member of this family, in the developing pituitary gland have demonstrated variable phenotypes mirroring that of *Hesx*$^{-/-}$ knockout mice, including the eye (microphthalmia, anophthalmia), forebrain (absent telencephalic vesicles) and pituitary (abnormal pituitary clefting, pituitary hyperplasia and ectopic pituitary tissue located in the roof of the oropharynx with absent posterior and intermediate lobes) [34]. While differentiation of hormone-producing cells appeared normal in these mutants, 25% of these mice exhibited dwarfism, indicating the possibility of hypothalamo-pituitary dysfunction. Additionally, expression of several early hypothalamo-pituitary signalling molecules including *Fgf8*, *Fgf10*, *Bmp4*, *Tbx2* and *Tbx3* was shifted anteriorly within the caudal hypothalamus, with a reduction in SHH expression. Further experiments with *Tcf7l1*$^{\Delta N/\Delta N}$ mice that lacked the β-catenin-interacting domain demonstrated normal pituitary development, indicating that the role of *Tcf7l1* as a transcriptional repressor is independent of β-catenin.

Terminal Cell Differentiation

Prop1

Prophet of PIT-1 (PROP1) is the earliest expressed pituitary-specific transcription factor. Its expression peaks at E12.0 and becomes undetectable by E15.5 [111, 112]. The onset of *Prop1* expression is required for the emergence of the *Pou1f1* lineage (somatotrophs, lactotrophs and thyrotrophs) [113–115], the activation of NOTCH2 with emergence of the gonadotroph lineage [115–117] and the repression of *Hesx1* and *Otx2* [97]. Its premature expression leads to agenesis of the anterior pituitary, probably by repression of *Hesx1*, while its persistence delays the differentiation of gonadotrophs [118, 119]. By expressing a constitutively active form of β-catenin throughout the pouch, Olson et al. [97] showed that the interaction between β-catenin and PROP1 mediates this dual role of concomitant repression of *Hesx1* and activation of *Pou1f1*. In humans, mutations in *PROP1* are the commonest genetic cause of combined pituitary hormone deficiency (CPHD) [76, 120, 121]. Recessive mutations are associated with GH, PRL and TSH deficiencies in addition to ACTH and gonadotropin deficiencies that may be present at a young age or develop over time [76–79, 122–128]. The anterior pituitary may be hypoplastic or enlarged and the size can wax and wane over time [79, 80, 128].

Pou1f1

POU1F1 (previously known as PIT-1) is a pituitary-specific transcription factor belonging to the POU homeodomain family. It is expressed late during pituitary development (E13.5) and its expression persists throughout adulthood [20, 129]. POU1F1 is important for (1) terminal differentiation and expansion of somatotrophs, lactotrophs and thyrotrophs, (2) repression of gonadotroph cell fate and (3) transcriptional regulation of the gene itself, suggesting that its autoregulation is required to sustain gene expression once the protein has reached a critical threshold [130–135]. In humans mutations in *POU1F1* are characterized by GH, TSH and PRL deficiencies [71, 136–138].

Other Late Transcription Factors Involved in Terminal Cell Differentiation

A number of transcription factors determine the gonadotroph cell fate (such as GATA2, SF1, EGR1, PITX1, PITX2 and PROP1) resulting in mature cells expressing terminal cell differentiation markers such as the GnRH receptor (GnRHR) and the hormone-specific β-subunit of LH (LHβ) and FSH (FSHβ) [9]. Steroidogenic factor 1 (*Sf1*) is expressed in gonadotrophs and the ventromedial hypothalamus as well as in the developing gonads and adrenal glands [9, 139–142]. It is a zinc finger nuclear receptor that regulates a number of genes including *Cga* (producing the protein α-GSU, the common subunit of LH, FSH and TSH), *Lhβ*, *Fshβ* and *Gnrhr* [139–143]. Pituitary-specific inactivation of *Sf1* results in mice with hypoplastic gonads, a dramatic decrease in pituitary gonadotropin expression and failure to develop normal secondary sexual characteristics. The adrenal glands and hypothalamus are unaffected [144].

The function of gonadotrophs in the anterior pituitary is under the control of hypothalamic GnRH synthesized by neurons in the preoptic nucleus, which project axons to the median eminence where the hormone is secreted. GnRH cells arise from the olfactory placode, and *Pax6* is required for their generation [145]. Mice with *Pax6* mutations show failure to develop both optic and olfactory placodes [146]. Following their generation, GnRH neurons migrate along the olfactory nerve pathway across the cribriform plate towards the olfactory bulb and their final position in the hypothalamus. In humans, it is estimated that migration of GnRH cells begins during the sixth week of gestation [147]. An increasing number of genes are implicated in the migration and maturation of GnRH neurons (*KAL1*, *FGFR1*, *FGF8*, *PROKR2*, *KISS1*, *GPR54*, *LEP*, *CHD7*, *TAC3*, *TACR3*) and their role is highlighted by mutations found in patients with isolated hypogonadotropic hypogonadism [148, 149].

Although pituitary corticotrophs are the first cell type to reach terminal differentiation, relatively little is known about the factors that determine the specification of corticotrophs and melanotrophs and the control of POMC expression. Corticotrophs start to differentiate at E12.5, producing POMC [18, 21]. The transcription factor *Tbx19* (*T-pit*) is expressed in corticotrophs and melanotrophs and, along with *Pitx1*, it activates the *Pomc* promoter [21, 150, 151]. More recently *Pax7* has been identified as an important factor in determining the identity of melanotrophs. *Pax7* inactivation results in loss of melanotroph gene expression and switching of cells to a corticotroph fate [152].

Congenital Disorders of Hypothalamo-Pituitary Development

Congenital hypopituitarism may arise from mutations in any of the genes involved in pituitary development and has a reported incidence of 1 in 3000–4000 births [153]. It is a highly heterogeneous disorder that may manifest either as an isolated hormone deficiency, the commonest being isolated growth hormone deficiency (IGHD), or as CPHD when two or more pituitary hormones are affected. The clinical features vary in severity and time of presentation; its onset may be early in the neonatal period or later in life (Table 5.5). Congenital hypopituitarism may also be part of a syndrome where abnormalities in extra-pituitary structures that share a common embryologic origin with the pituitary gland (such as eye, midline and forebrain abnormalities [Table 5.3]) occur in addition to pituitary hormone deficiencies. The aetiology remains unknown in the majority of patients.

Table 5.5 Symptoms and signs in patients with congenital hypopituitarism.

Endocrine-related symptoms and signs	
Hypoglycaemia, jitteriness	
Apnoea	
Prolonged jaundice, conjugated hyperbilirubinaemia	
Temperature instability	
Poor feeding and weight gain	
Recurrent sepsis	
Lethargy	
Electrolyte abnormalities (hyponatraemia without hyperkalaemia, hypernatraemia)	
Growth failure/deficit	
Pubertal delay	
Genital appearances	
Micropenis	
Bilateral undescended testes	
Macroorchidism (adults with *IGSF1* mutation)	
Midline defects and craniofacial abnormalities	
Cleft lip and palate	
Single central incisor	
Holoprosencephaly	
Absent septum pellucidum	
Absent corpus callosum	
Eye defects	
Anophthalmia/severe bilateral microphthalmia	
Optic nerve hypoplasia	
Retinal dysplasia	
Nystagmus	
Variable syndromes associated with hypopituitarism	
AEG	Anophthalmia, oesophageal atresia, genital abnormalities
PHACE(S)	Posterior fossa malformations (e.g. Dandy–Walker cysts), haemangioma (face and neck), arterial malformations, cardiac defects, eye abnormalities, sternal defects (clefts, supraumbilical raphe)
Rieger	Malformations of anterior chamber of the eye, protuberate umbilicus, abnormal dentition, mental retardation
Johanson–Blizzard	Microcephaly, pancreatic exocrine insufficiency, recto-urethral abnormalities, primary hypothyroidism
Pallister–Hall	Polydactyly, imperforate anus, hypothalamic hamartoblastoma

Combined Pituitary Hormone Deficiencies (CPHD)

Non-syndromic CPHD

PROP1 Mutations

PROP1 (OMIM 601538) consists of three exons encoding a 226-amino-acid protein. *PROP1* mutations are the commonest cause of CPHD accounting for ~50% of familial cases. The first mutations were identified in four unrelated pedigrees with hypopituitarism characterized by GH, TSH, PRL and gonadotropin deficiencies with

failure to enter puberty [154]. Subsequent mutations were reported in pedigrees from different countries, with a much lower incidence in sporadic cases [76, 80, 120, 121]. The majority of the reported homozygous mutations involve the highly conserved DNA-binding homeodomain resulting in complete or partial loss of function of the mutant proteins by ablating DNA binding and transcriptional activation [154, 155].

The most common mutation (50–72% of all familial *PROP1* mutations) is a two-base-pair deletion within exon 2 leading to a truncated protein (S109X) [76, 120, 156]. The deletion occurs within three tandem GA repeats, so the two base pairs deleted cannot be defined; this has been referred to as c.296delGA and c.301_302delAG in different reports. It is likely to represent a mutational hotspot within the gene, rather than a common founder mutation [120]. Along with c.150delA, this mutation accounts for ~97% of all mutations in *PROP1*.

Homozygous *PROP1* mutations are typically associated with GH, TSH, PRL and gonadotropin deficiencies but the time of onset and severity of hormone deficiencies varies, even between siblings carrying identical mutations, which suggests that as yet unidentified genetic or epigenetic modifying factors may play a role in the pathogenesis. Most patients present with early-onset GH deficiency and growth retardation but normal growth velocity in early childhood has been reported in a patient who attained normal adult height without GH replacement therapy. In this case, the patient presented with gonadotropin deficiency with the evolution of other hormone deficiencies later in life, including GH. Normal adult height was achieved at the expense of considerable weight gain at the time of puberty [123, 157].

TSH deficiency may be the initial presenting symptom or present later in life [76–79]. The onset of ACTH deficiency is correlated with increasing age, most patients having normal ACTH and cortisol concentrations in early life, with evolution of deficiency over time but cortisol deficiency has been described in a 7-year-old patient, emphasizing the necessity for continuing clinical assessment of patients with *PROP1* mutations [122, 124–128]. The underlying mechanism for cortisol deficiency remains unknown, especially as *PROP1* is not expressed in corticotrophs, but it is required for the maintenance of the corticotroph population.

Although *PROP1* plays a critical role in the differentiation of gonadotrophs in the mouse, the spectrum of gonadotropin deficiency is extremely variable in humans and ranges from hypogonadism presenting with microphallus and undescended testes to lack of pubertal development. This can be spontaneous, although often delayed, and gonadotropin deficiency may become manifest later with pubertal arrest and infertility [76–78, 80, 127, 129]. Variations in the timing and severity of gonadotropin deficiency suggest a role for *PROP1* in the maintenance and terminal differentiation of gonadotrophs rather than their initial development [158]. The evolving nature of hormone deficiencies suggests a progressive decline in the function of the anterior pituitary, indicating a need for continual monitoring for the development of hormone deficits that may not be apparent initially.

Pituitary morphology also varies. Most patients have a small or normal anterior pituitary with a normal posterior lobe and pituitary stalk but an enlarged anterior pituitary has also been reported and the size of the pituitary can wax and wane with subsequent involution in older patients (Figure 5.5) [79, 80, 128]. The pituitary enlargement consists of a mass lesion interposed between the anterior and posterior lobes, possibly originating from the intermediate lobe, but the underlying mechanism remains unknown [79]. Studies suggest that the fetal pituitaries of PROP1-deficient mice retain mutant cells in the periluminal area of Rathke's pouch that fail to differentiate, exhibiting enhanced apoptosis and reduced proliferation [117]; this would provide a possible explanation for the human imaging findings.

POU1F1 Mutations

POU1F1 (OMIM 173110) consists of six exons encoding a 291-amino-acid protein. POU1F1 has three functional domains: a transactivation domain, a POU-specific domain and a POU homeodomain. Both POU domains are critical for high affinity DNA binding on GH and PRL promoters [129, 159, 160].

The first *POU1F1* mutations in association with GH, PRL and TSH deficiencies and variable pituitary hypoplasia were reported by four independent groups [71, 136–138]. The incidence of *POU1F1* mutations is low in cases of sporadic CPHD (3–6%) and higher in familial cases (25%) [72, 161]. Functional analysis suggests that some mutations disrupt DNA binding, whereas others disrupt transcriptional activation or other properties such as autoregulation [72, 162, 163]. The majority of mutations are recessive (e.g. p.R172X, p.A158P, p.P239S, p.E230K) but the heterozygous p.R271W mutation appears to be a 'hotspot' for mutations within *POU1F1* and has been identified in several unrelated patients from different ethnic backgrounds [136, 137, 164–170]. This mutant protein is capable of binding to DNA and appears to act as a dominant negative inhibitor of transcription by wild-type POU1F1 protein [137, 171].

Patients with *POU1F1* mutations present early in life with GH and PRL deficiencies. TSH deficiency can be highly variable, the majority of patients presenting early, but hypothyroidism has also been reported later in childhood [71, 172, 173]. A *POU1F1* mutation was reported

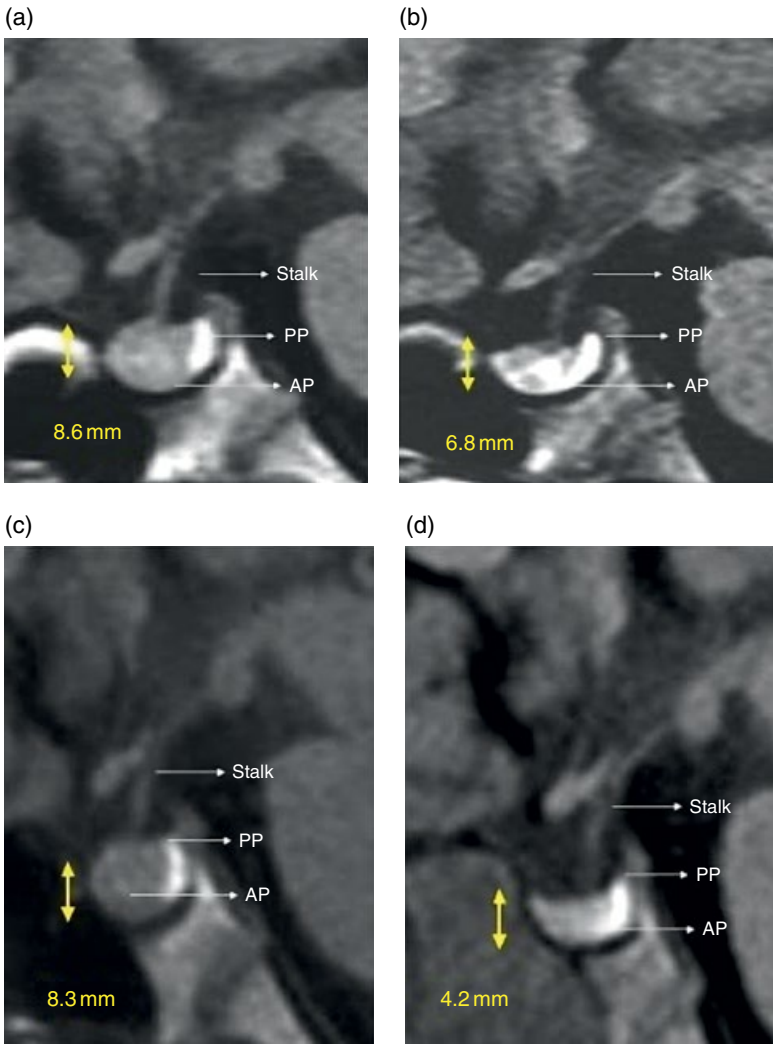

Figure 5.5 MR imaging of a 9-year-old boy with a 13-base-pair deletion in *PROP1* showing (a) an enlarged sella turcica with a markedly enlarged anterior pituitary (AP 8.6 mm), with enhancing lesions suggestive of possible haemorrhage at initial presentation; (b) an anterior pituitary size of 6.8 mm four months later; (c) an anterior pituitary size of 8.3 mm 12 months later; and (d) an anterior pituitary size of 4.2 mm 21 months later.

in a 21-year-old with GH and PRL deficiency who had normal thyroid function and yet carried an identical mutation to an unrelated patient who developed central hypothyroidism in the second year of life, suggesting that other genetic or environmental factors may modify the onset of TSH deficiency [72]. Patients with *POU1F1* mutations have a small- or normal-sized anterior pituitary gland with a normal posterior pituitary and infundibulum and no extra-pituitary abnormalities [9].

Syndromic CPHD

Septo-Optic Dysplasia (SOD) and its Variants

SOD is a rare congenital heterogeneous disorder involving forebrain, eye and pituitary abnormalities with a prevalence ranging from 6.3 to 10.9 per 100,000 [174]. It is defined by at least two of optic nerve hypoplasia (ONH), midline forebrain defects (e.g. agenesis of the corpus callosum, absent septum pellucidum), and pituitary hypoplasia with variable hypopituitarism (Figure 5.6) [175]. Thirty percent of patients have the complete triad, 60% have an absent septum pellucidum and 62% have variable hypopituitarism [176].

ONH may be unilateral (12%) or bilateral (88%) and may be the first presenting feature, with development of pituitary hormone deficiencies later in life [176, 177]. There appears to be little correlation between the size of the optic nerve and visual function but ocular abnormalities may be more severe and include bilateral anophthalmia or severe microphthalmia. Associated features include cavum septum pellucidum, cerebellar hypoplasia, schizencephaly and aplasia of the fornix. Neurological manifestations are common (75–80%) and range from focal deficits to global developmental delay [177].

75–80% of patients with ONH have neuroradiological abnormalities; anterior pituitary hypoplasia, an undescended (ectopic) posterior pituitary and an absent pituitary stalk are predictors of hypopituitarism. In our series,

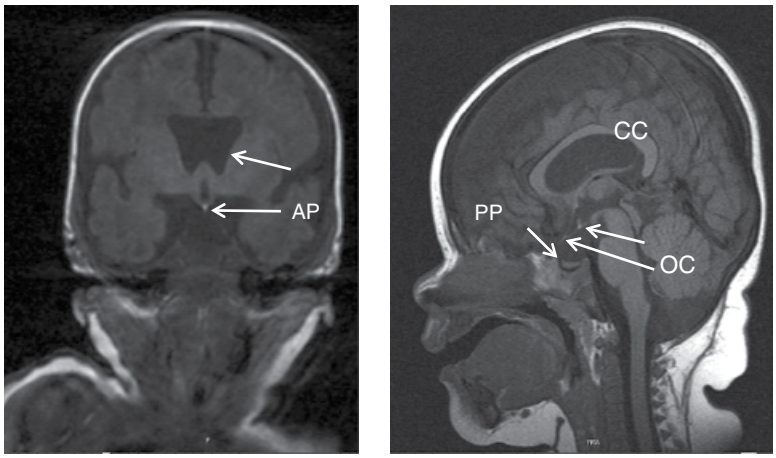

Figure 5.6 MR imaging of a patient with septo-optic dysplasia demonstrating the absence of septum pellucidum (SP) dividing the lateral ventricle, a thin corpus callosum (CC), anterior pituitary (AP), hypoplasia and ectopic posterior pituitary (PP). The optic chiasm (OC) is also thin, suggesting the presence of hypoplasia of the optic pathways.

the risk of hypopituitarism was 27.2 times greater in patients with an undescended posterior pituitary as compared with those with a eutopic posterior pituitary (Figure 5.7) [178]. Pituitary hypoplasia may manifest as endocrine deficits varying from IGHD to panhypopituitarism. The commonest endocrinopathy is GH deficiency followed by TSH and ACTH deficiencies, while gonadotropin secretion may be retained. Endocrinopathies may evolve over time [177].

The aetiology of SOD is multifactorial, and both genetic and environmental factors (viral infections, vascular or degenerative changes, exposure to alcohol or drugs) are implicated. SOD presents more commonly in children born to younger mothers and as clusters in geographical areas with a high frequency of teenage pregnancies [95, 175]. Since the development of the forebrain and pituitary are closely linked, both occurring at 3–6 weeks of gestation, any insult at this stage of development could account for SOD and its variants.

During these stages signalling molecules and transcription factors have extensive and overlapping patterns of expression in the prospective forebrain, pituitary and sensory placodes and contribute to the development of the hypothalamo-pituitary axis at multiple levels, directly or indirectly. It is not surprising, therefore, that there is increasing evidence of overlap in the aetiology of conditions that were previously considered to be discrete, such as SOD, CPHD and Kallmann syndrome because mutations in the same array of genes (*KAL1*, *PROKR2*, *FGF8*, *FGFR1*) have been implicated in their aetiology [64, 65, 67]. For example, in humans, heterozygous or recessive *FGF8* mutations have been reported in association with Kallmann syndrome [179, 180] but heterozygous *FGF8* mutations (p.Q216E) have been reported in association with SOD whilst an autosomal recessive case of holoprosencephaly (HPE) has also been attributed to *FGF8* (p.R189H) [64].

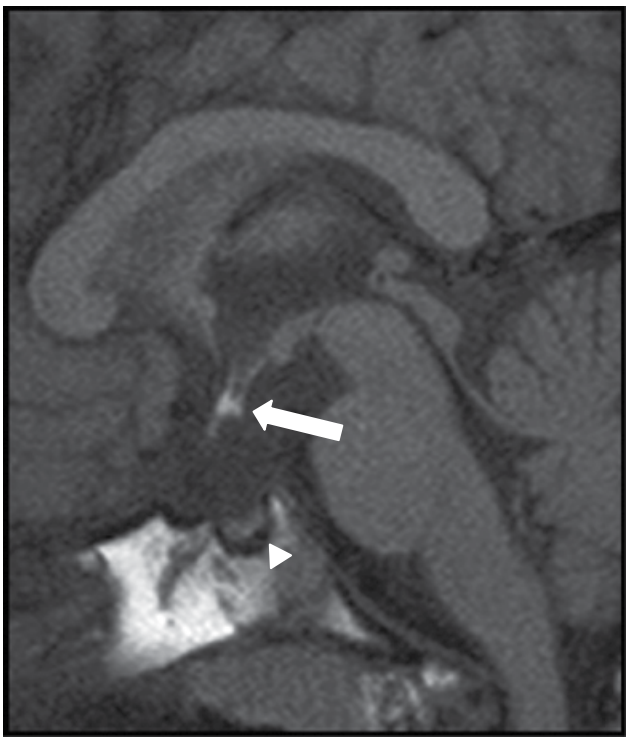

Figure 5.7 Sagittal MRI with dedicated pituitary views demonstrating the presence of anterior pituitary hypoplasia (arrowhead) with an undescended, ectopic posterior pituitary (arrow) and an absent pituitary stalk in a patient with GH deficiency.

HESX1 Mutations

HESX1 (OMIM 601802) maps to chromosome 3p21.1–21.2, and its coding region spans 1.7 kb with a highly conserved genomic organization consisting of four coding exons [30]. In humans, the first homozygous *HESX1* mutation (p.R160C) was described in two siblings from a highly consanguineous pedigree presenting with hypopituitarism in association with ONH, absence of the corpus callosum, anterior pituitary hypoplasia and an ectopic posterior

Table 5.6 *HESX1* mutations and their associated phenotypes.

Mutation	Inheritance	Endocrine phenotype	MRI phenotype
p.Q6H	AD	GH, TSH, LH, FSH, evolving ACTH deficiency	APH, EPP
p.Q117P	AD	GH, TSH, LH, FSH, ACTH deficiency	APH, EPP
p.E149K	AD	GH deficiency	APH, EPP, infundibular hypoplasia
p.S170L	AD	GH deficiency	Normal AP, EPP, ONH, partial ACC
p.K176T	AD	GH, evolving ACTH and TSH deficiency	EPP
p.T181A	AD	GH deficiency	APH, absent PP bright spot, normal ON
g.1684delG	AD	GH deficiency	APH, absent PP bright spot, ONH, ACC
c.306_307insAG	AD	GH, TSH, LH, FSH deficiency	APH, ONH
p.R160C	AR	GH, TSH, LH, FSH, ACTH deficiency	APH, EPP, ONH, ACC
p.I26T	AR	GH, LH, FSH, evolving ACTH and TSH deficiency	APH, EPP, normal ON
c.357+2T>C	AR	GH, TSH, ACTH, PRL deficiency	APH, normal PP and ON
Alu insertion (exon 3)	AR	Panhypopituitarism	APH, normal PP and infundibulum
c.449_450delCA	AR	GH, TSH, ACTH deficiency	AP aplasia, normal PP and ON, thin CC, hydrocephalus

ACC, agenesis of the corpus callosum; AD, autosomal dominant; AP, anterior pituitary; APH, anterior pituitary hypoplasia; AR, autosomal recessive; CC, corpus callosum; EPP, ectopic posterior pituitary; ON, optic nerve; ONH, optic nerve hypoplasia; PP, posterior pituitary.

pituitary [30]. This missense mutation was found in the homeobox of *HESX1* of the two siblings, while the phenotypically normal parents were heterozygous.

This resulted in a complete loss of DNA binding and the mutation was unusually associated with an *in vitro* dominant negative effect even though heterozygotes for the mutation did not manifest the phenotype. Subsequently, homozygous and heterozygous *HESX1* mutations have been described in patients with highly variable phenotypes without obvious genotype–phenotype correlation [9, 175]. Heterozygous mutations (p.E149K, p.S170L, p.T181A, g.1684delG) can be associated with IGHD in patients exhibiting a relatively milder phenotype compared with the severe manifestations of SOD; these patients may or may not have ONH, an ectopic posterior pituitary or anterior hypoplasia [27, 181, 182]. *HESX1* mutations are an uncommon cause of hypopituitarism and SOD, accounting for <1% of cases (Tables 5.3 and 5.6) [182].

TCF7L1 Variants

Two variants of *TCF7L1* associated with forms of SOD in humans have been discovered [34]. The first, p.R92P, was present in a male infant with partial agenesis of the corpus callosum, thin anterior commissure, right optic tract hypoplasia and marginally low IGF-1 concentrations. The variant was also present in his father and paternal uncle who were asymptomatic. A second male infant with bilateral ONH, anterior pituitary hypoplasia, absent septum pellucidum and posterior pituitary bright spot was found to have the second variant, p.R400Q, which was present in his unaffected mother and two siblings. Both variants showed reduced *TCF7L1* repressing activity *in vitro* and in an *in vivo* zebrafish model, suggesting that they exhibited variable penetrance in humans.

Congenital Hypopituitarism and Severe Ocular Abnormalities

SOX2 *SOX2* (OMIM 184429) is a single exon gene located on chromosome 3q26.3-27, which encodes a 317-amino-acid protein [183]. Heterozygous *de novo* loss-of-function *SOX2* mutations were initially described in patients with bilateral anophthalmia or severe microphthalmia and have been detected in 10–20% of these individuals [43, 44, 184, 185]. Other abnormalities include developmental delay, spastic diplegia, epilepsy, oesophageal atresia, tracheo-oesophageal fistula, sensorineural hearing loss and male genital abnormalities [43, 185, 186].

The association of the loss of *SOX2* with hypopituitarism was first reported in a cohort of patients with severe eye abnormalities (anophthalmia, bilateral microphthalmia) with or without other developmental defects [43]. All patients had isolated gonadotropin deficiency, anterior pituitary hypoplasia and midline or forebrain defects, suggesting a clinical phenotype within the SOD spectrum. Apart from anterior pituitary hypoplasia, other abnormalities include generalized defects in white matter, hippocampal hypoplasia, rotated mesial temporal structures, hydrocephalus or cystic dilatation of a single ventricle, hypothalamic hamartoma (or slowly

progressing hypothalamo-pituitary tumours), absent septum pellucidum and hypoplasia, dysgenesis or partial agenesis of the corpus callosum.

Hypogonadotropic hypogonadism is a constant finding in these patients and GH insufficiency may be observed in some cases [44, 45, 187]. The apparent selectivity for gonadotropin deficiency and the sparing of other hormone axes in the face of pituitary hypoplasia suggest that *SOX2* is involved independently at multiple levels during the development of the hypothalamo-pituitary axis. In fact, patients with inactivating *SOX2* mutations (p.P79L, c.60insG) and hypogonadotropic hypogonadism exhibit an increase in LH and FSH after sustained GnRH stimulation [110], which suggests that there are functional gonadotrophs that are able to receive and respond to the GnRH signal even within a hypoplastic gland.

Although the majority of patients have severe bilateral ocular manifestations, the phenotype can be less severe as in the case of a young female with a heterozygous loss-of-function mutation within the HMG domain (p.L75Q) who had isolated hypogonadotropic hypogonadism but a normal anterior pituitary and unilateral rather than bilateral anophthalmia [45]. Not all patients with *SOX2* mutations have developmental delay or display the full spectrum of extra-pituitary abnormalities. This could result from the redundant function of other SoxB1 transcription factors or a differential sensitivity to the level of *SOX2* expression in different areas of the developing CNS and other tissues [188, 189].

OTX2 Orthodenticle homeobox 2 (*OTX2*, OMIM 600037) is a transcription factor located on chromosome 14q22 required for the formation of anterior structures and maintenance of the forebrain [190]. It has been implicated in 2–3% of anophthalmia or microphthalmia in humans [191–193]. Homozygous knockout mice die at mid-gestation with severe brain abnormalities, whereas heterozygous mutants have a variable phenotype ranging from normal to severe eye and brain abnormalities (anophthalmia, HPE or anencephaly) [194–196]. *Otx2* transcripts are detected from E10.5 in the ventral diencephalon and Rathke's pouch but, by E12.5, expression persists only in the ventral diencephalon. From E16.5 and postnatally, *Otx2* expression is undetectable in both sites [197].

Heterozygous *OTX2* mutations have been described in patients with variable ocular malformations (anophthalmia, microphthalmia, retinal degeneration) and, even more rarely, in patients with no eye abnormality [53, 54, 191, 193, 198, 199]. The pituitary phenotype ranges from partial to complete GH deficiency or hypopituitarism with or without an ectopic posterior pituitary on MR imaging (MRI). There is no clear genotype–phenotype correlation, even among patients with the same mutation (Table 5.7). Due to the relative short-term follow-up data in these reports, one cannot exclude the development of other pituitary hormone deficiencies over time.

Table 5.7 *OTX2* mutations and their associated phenotypes.

Mutation	Mutation type	Endocrine phenotype	MRI phenotype	Other associations
p.R90S	Missense	IGHD	APH, EPP	Unilateral anophthalmia, learning difficulties
p.S138X	Nonsense	Low IGF-1 and IGF-BP3	Normal	Retinal dystrophy, failure to thrive
p.G188X	Nonsense	GH, LH, FSH, TSH, PRL deficiency	APH, EPP	Bilateral microphthalmia
		No deficit	Not available	Bilateral microphthalmia, seizures
c.221_236del15	Frameshift	IGHD	APH, EPP	Right anophthalmia, left microphthalmia, developmental delay
p.N233S[a]	Missense	GH, LH, FSH, TSH, ACTH deficiency	APH, EPP	Normal eyes
			APH	Normal eyes
c.402_403insC	Frameshift	Partial GH deficiency	Normal	Bilateral anophthalmia, developmental delay, cleft palate
c.576_577insCT[b]	Frameshift	GH, LH, FSH, TSH, ACTH deficiency	APH, EPP, Chiari malformation	Bilateral anophthalmia, developmental delay
OTX2 gene deletion		IGHD	APH	Left anophthalmia, right microphthalmia

APH, anterior pituitary hypoplasia; EPP, ectopic posterior pituitary; IGHD, isolated growth hormone deficiency.
[a] In subsequent reports referred to as p.N225S when a different transcript is used for nomenclature.
[b] In subsequent reports referred to as c.404_405insCT when a different transcript was used for nomenclature.

Congenital Hypopituitarism with or Without Mental Retardation

SOX3 (OMIM 313430) is a single exon gene that maps to chromosome Xq27 and encodes a transcript that consists of a short N-terminal domain, a 79-amino-acid DNA-binding HMG domain and a longer C-terminal domain containing four polyalanine stretches involved in transcriptional activation [200, 201]. *SOX3* has been implicated in the aetiology of X-linked hypopituitarism with a highly variable phenotype in patients with over- and underdosage of *SOX3* presenting with IGHD or CPHD with or without mental retardation or learning difficulties [48, 202–204]. Most patients have anterior pituitary hypoplasia and an ectopic posterior pituitary but patients with altered *SOX3* dosage have also been reported to have a eutopic posterior pituitary or additional abnormalities including a persistent craniopharyngeal canal (Table 5.8) [49].

Large (3.9–13 Mb) [203, 205–207] or submicroscopic (685.6 kb) [48] duplications encompassing *SOX3* have been associated with hypopituitarism. In addition, expansion of the first polyalanine tract by seven or eleven residues or an in-frame deletion resulting in the loss of six alanine residues has been reported in association with variable phenotypes (IGHD or CPHD) [48, 50]. *In vitro* experiments have shown a variable mechanism of action. Polyalanine tract expansions result in reduced transcriptional activation of target genes due to retention of the mutant protein in the cytoplasm with failure to translocate to the nucleus [48]. Polyalanine tract deletions result in increased transcriptional activation *in vitro* and this may be comparable with increased dosage of the gene as observed with genetic duplications [51]. In any case, variability in the size of the *SOX3* polyalanine tract is an uncommon cause of hypopituitarism or IGHD and

Table 5.8 SOX3 dosage changes and their associated phenotypes.

Dose change	Endocrine phenotype	MRI phenotype	Other associations
Deletions			
2.31 Mb	GH and gonadotropin deficiency, low-normal fT_4 and TSH	Persistent craniopharyngeal canal, small AP, normal PP	Haemophilia B, developmental delay
Duplications			
3.9–13 Mb	GH deficiency, variable ACTH, TSH and gonadotropin deficiency	Not available	Variable developmental delay, mental retardation, spina bifida
7.5 Mb	Short stature, hypopituitarism	Not available	Affected females; facial dysmorphism, hearing and speech impairment
6 Mb	Growth retardation, scrotal hypoplasia	Not available	XX male sex reversal, developmental delay, microcephaly
685.6 kb	GH deficiency, evolving TSH deficiency	APH, EPP	No mental retardation or craniofacial dysmorphism
PA tract expansion			
+11 PA	GH deficiency	Not available	Mental retardation, facial abnormalities in some patients
+7 PA	Panhypopituitarism	APH, EPP	No mental retardation, no facial dysmorphism
+7 PA	GH deficiency	APH, EPP	With/without mental retardation in same family
PA tract deletions			
del6PA	GH, TSH and gonadotropin deficiency	Cyst-like AP appearance, normal PP	Affected female; normal development
del9PA	None reported	Not available	Mental retardation

AP, anterior pituitary; APH, anterior pituitary hypoplasia; EPP, ectopic posterior pituitary; PA, polyalanine; PP, posterior pituitary.

there have been no reported point mutations leading to functional compromise.

Holoprosencephaly

HPE is a developmental abnormality of the forebrain with an incidence of 1 in 10,000–20,000 in the general population [208, 209]. It is defined by varying degrees of defects in the separation of the cerebral hemispheres and ventricles, while failure of the frontal and parietal lobes to divide posteriorly results in an absent corpus callosum. Variable midline facial defects are associated with this condition, ranging from cyclopia or anophthalmia to midfacial hypoplasia, hypotelorism, cleft lip and/or palate and a single central incisor. The most common pituitary abnormality is diabetes insipidus (DI), although anterior pituitary hormone deficiencies have also been described.

Cytogenetic abnormalities are present in about 25% of patients with HPE [208, 210]. Of those with normal cytogenetics, only a further 17% have identifiable mutations. This suggests that a number of genetic factors have yet to be described. An increasing number of genes are implicated in its aetiology, including members of the SHH signalling pathway such as *SHH*, *ZIC2*, *TGIF1*, *PTCH1*, *GLI2*, *DISP1*, *TDGF1*, *GAS1*, *EYA4* and *FOXH1* [208]. Heterozygous mutations in *GLI2* have been reported in patients with variable craniofacial abnormalities, IGHD or panhypopituitarism and abnormal pituitary morphology (absent pituitary or hypoplasia) [58]. Mutations in *FGF8* have also been identified as the first genetic cause of autosomal recessive HPE [64].

Congenital Hypopituitarism with Neck or Cerebellar Abnormalities

LHX3 (OMIM 600577) Patients with homozygous or compound heterozygous *LHX3* mutations present with GH deficiency in association with TSH, gonadotropin or PRL deficiency, with or without ACTH deficiency [35–37]. Although ACTH secretion is preserved in most patients, early-onset ACTH deficiency has been reported in a patient who presented with severe early-onset hypopituitarism, neonatal hypoglycaemia and low random cortisol concentrations, with an impaired response to ACTH stimulation [37]. MRI may show a normal, hypoplastic or enlarged anterior pituitary, the latter even occurring 10 years after a previously normal scan [35] or in one case demonstrating hypointensity consistent with a microadenoma [38]. Other associated features include a short neck with limited rotation, spinal abnormalities, loose skin and variable sensorineural hearing loss [36, 37].

LHX4 (OMIM 602146) Until recently heterozygous *LHX4* mutations have been reported only in patients with GH deficiency and short stature on presentation in association with variable endocrine deficits (ACTH, TSH, gonadotropin deficiency) or panhypopituitarism [39–41, 211]. However, there is remarkable variability in the phenotype even within the same family, ranging from panhypopituitarism to GH deficiency with partial TSH deficiency or partial GH deficiency and short stature diagnosed in adulthood with no additional hormone deficiencies. Neuroradiological findings include a hypoplastic, normal or enlarged anterior pituitary, an ectopic posterior pituitary (in almost a third of cases), a hypoplastic corpus callosum and Chiari malformation. Neck rotation and hearing are normal.

The first homozygous missense *LHX4* mutation (p.T126M) was reported in two deceased male patients of Pakistani origin with severe panhypopituitarism, anterior pituitary aplasia and an ectopic posterior pituitary [42]. Both siblings were born small for gestational age with a small phallus, undescended testes and midfacial hypoplasia and developed severe respiratory distress postnatally. Despite rapid commencement of hydrocortisone and thyroxine, both died within the first week of life. The severity of the respiratory compromise mirrors the mouse model, in which null mutants die within the first week of life from immature lungs that fail to inflate.

PNPLA6 (OMIM 603197) *PNPLA6* encodes neuropathy target esterase (NTE), a lysophospholipase that is expressed in neurons throughout the brain, including the cortex and Purkinje cells of the cerebellum. Loss-of-function *PNPLA6* mutations have been reported in patients with progressive cerebellar ataxia or atrophy, chorioretinal dystrophy and variable pituitary dysfunction including GH and TSH deficiencies and normosmic hypogonadotropic hypogonadism, the latter manifesting as pubertal failure. These findings suggest a genetic overlap between the hitherto unexplained Oliver–McFarlane and Laurence–Moon syndromes [68].

Other Syndromic Forms

Mutations within *PITX2* (OMIM 601542) are associated with Axenfeld–Rieger syndrome. Rieger syndrome is a heterogeneous autosomal dominant condition with variable manifestations including anomalies of the anterior chamber of the eye, dental hypoplasia, a protuberant umbilicus, mental retardation and pituitary abnormalities. All mutations identified within *PITX2* to date are heterozygous,

Table 5.9 Genetic forms of isolated GH deficiency (IGHD).

Type	Inheritance	Phenotype	Gene(s)	Mutations
IA	AR	Undetectable GH; anti-GH antibodies on rhGH treatment	*GH1*	Deletions (6.7, 7.0, 7.6, 45 kb) Frameshift Nonsense
IB	AR	Low, detectable GH; no antibodies on rhGH treatment	*GH1 GHRHR* ?other	Splice site Missense Nonsense Frameshift
II	AD	Variable short stature (severe to normal height); may have evolving endocrine deficits	*GH1*	Splice site Splice site enhancers Missense
III	XL	IGHD or in combination with other hormone deficiencies; EPP ± mental retardation	*SOX3*	PA tract deletions/expansions Gene deletions/duplications
		±agamma/hypogammaglobulinaemia	*HTK*, unknown	

AD, autosomal dominant; AR, autosomal recessive; EPP, ectopic posterior pituitary; PA, polyalanine; rhGH, recombinant human GH.

affecting the homeodomain of the gene. Some patients have reduced GH concentration and anterior pituitary hypoplasia, while others may have an abnormal sella turcica without endocrine dysfunction [62, 63]. Although these observations suggest a role for *PITX2* in pituitary development, its importance and contribution remain to be identified.

Homozygous mutations in *ARNT2* (OMIM 606036) (c.1373_1374dupTC), a gene implicated in hypothalamic development, have been reported in six affected children of a highly consanguineous family [57]. All patients presented in the first month of life with hypernatraemia secondary to central DI and cortisol insufficiency. Some children presented with or subsequently developed central hypothyroidism and/or demonstrated an abnormal pattern of growth, with either growth failure or maintenance of linear growth in conjunction with obesity. In this case, the multiple pituitary hormone deficiencies were associated with postnatal microcephaly, frontotemporal lobe hypoplasia, seizures, severe visual impairment and abnormalities of the kidneys and urinary tract. MRI revealed an absent posterior pituitary with a thin pituitary stalk and anterior pituitary hypoplasia as well as a thin corpus callosum and a global delay in brain myelination.

Isolated Pituitary Hormone Deficiencies

Isolated Growth Hormone Deficiency (IGHD)

Congenital IGHD is the commonest pituitary hormone deficiency with an incidence of 1 in 4,000–10,000 live births. Although most cases are sporadic, 3–30% are familial [212–215]. IGHD has been classified into four genetic types depending on the pattern of inheritance: autosomal recessive (types IA and IB), autosomal dominant (type II) and X-linked recessive (type III) (Table 5.9) [216, 217]. Mutations are identified in up to 11% of patients with a higher prevalence in familial cases (34%) as compared with sporadic IGHD [218, 219]. The commonest genes implicated are those encoding GH (*GH1*) and the receptor for GHRH (*GHRHR*). IGHD can also result from heterozygous mutations in early developmental transcription factors of development of the anterior pituitary and somatotrophs (*HESX1*, *SOX3, SOX2*, *OTX2*). It may be the initial presentation before the development of multiple pituitary hormone deficiencies, as is the case with mutations in transcription factors involved in the later stages of pituitary cell differentiation such as *PROP1* or *POU1F1* [216].

GH1 and GHRHR

The *GH1* (OMIM 139250) gene is located on the long arm of chromosome 17 (17q22-24) within a cluster of five homologous genes [220]. It consists of five exons and its full-length product is a 191-amino-acid (22 kDa) peptide representing about 75% of the circulating GH [216, 217, 221]. Alternative splicing may result in complete skipping of exon 3 and the generation of a 17.5 kDa variant that lacks amino acids 32–71, representing 1–5% of circulating GH. Another product of aberrant splicing is the 20 kDa molecule that represents 5–10% of circulating GH.

The gene encoding GHRHR (OMIM 139191) maps to chromosome 7p15 and consists of 13 exons spanning ~15 kb [222]. It encodes a 423-amino-acid G-protein-coupled receptor comprising seven transmembrane domains. The

expression of *GHRHR* is upregulated by *POU1F1* and required for the proliferation of somatotrophs [223].

IGHD Caused by *GH1* Mutations Homozygous *GH1* deletions of various size were first described in families with early severe growth failure (height <–4.5 SDS), undetectable GH concentration and poor response to treatment because of the development of antibodies (type 1A IGHD) [217, 224–229]. Similar phenotypes can result from homozygous or compound heterozygous mutations that result in a severely truncated or absent GH molecule [219, 230–232]. On the other hand, patients with type 1B IGHD due to *GH1* mutations also have marked short stature with low but detectable GH concentrations and a good response to recombinant human GH (rhGH) treatment [216].

The commonest genetic form of IGHD is inherited in an autosomal dominant manner (type II) [218, 233, 234]. Patients vary in the age of presentation and the degree of growth failure from severe short stature to even normal height [235, 236]. They have low but detectable GH with or without anterior pituitary hypoplasia on MRI (35–80%) [236, 237]. There is substantial variation in the severity of GH deficiency and patients of the same pedigree having the same mutation (e.g. p.R183H) can vary considerably in height (≤–4 SDS to normal) and even attain normal adult height without treatment [235, 236].

Patients with autosomal dominant GH deficiency may develop additional pituitary hormone deficits including ACTH, TSH, PRL and gonadotropin deficiencies [221, 238, 239]. They require lifelong follow-up. The evolving phenotype can be explained by the mechanism by which heterozygous *GH1* mutations cause IGHD, since the 17.5 kDa isoform exerts a dominant negative effect on the production of the 22 kDa molecule [240, 241]. The 17.5 kDa molecule has a dose-dependent deleterious effect in *in vitro* and *in vivo* studies. It is retained in the endoplasmic reticulum and triggers a misfolded protein response and the accumulation of macrophages resulting in the disruption of the Golgi apparatus. The tracking and secretory pathways of GH and other hormones such as ACTH, TSH and LH are therefore impaired.

Another heterozygous *GH1* mutation (p.R77C) has been reported in a patient with growth retardation and delayed puberty who showed normal catch-up growth with rhGH replacement therapy but there is no clear phenotype–genotype correlation as the same mutation has also been identified in family members of normal height [242]. Patients with this mutation may have normal or slightly increased GH secretion and low IGF-1 and GH-binding protein (GHBP) concentrations. Functional studies do not show a difference between the mutant and wild-type GH molecule in terms of binding to the GH receptor and activation of the downstream JAK2/STAT5 pathway but it is possible that the mutation results in reduced capability to induce *GHR/GHBP* gene transcription compared with the wild-type molecule.

IGHD Caused by *GHRHR* Mutations *GHRHR* mutations were first reported in two patients of a consanguineous pedigree who had severe IGHD resulting from the homozygous p.E72X *GHRHR* mutation that caused premature termination and a truncated protein missing all transmembrane domains of the receptor [243]. A number of homozygous or compound heterozygous mutations have since been reported (missense, nonsense, splice site, deletions or regulatory mutations) leading to autosomal recessive type IB IGHD [244–256]. Patients are usually of consanguineous pedigrees and from specific ethnic backgrounds including the Indian subcontinent, Pakistan, Sri Lanka, Somalia and Brazil [257–259].

Children usually have severe growth failure with height up to –7.4 SDS, undetectable GH concentrations, a blunted GH response to various stimuli, low IGF-1 and IGF-BP3 and a good response to treatment with rhGH. Compared with patients with recessive *GH1* mutations, midfacial hypoplasia, neonatal hypoglycaemia and microphallus are uncommon [216]. Recently, a 2-year-old child from a consanguineous pedigree with severe short stature (–5 SDS) was reported to present with hypoglycaemia leading to convulsions [260]. GH deficiency was subsequently diagnosed and the child was found to be homozygous for the p.C64G mutation in exon 3.

Due to the role of GHRH in the proliferation of somatotrophs, the finding of anterior pituitary hypoplasia on MRI has been considered to be almost invariable in these patients [216]. A number of reports suggest variability of anterior pituitary size, even among family members having the same *GHRHR* mutation. This finding may be explained by the fact that patients were of different ages and by the lack of well-defined age-matched reference standards [244]. With regard to the mechanism of action, mutations in *GHRHR* may impair ligand binding and signal transduction or affect the trafficking and localization of the receptor to the cell membrane [216]. A heterozygous change in *GHRH* has recently been reported in patients with sporadic IGHD [247]. Although a digenic effect cannot be excluded in these patients, it has been proposed that this may represent a novel form of IGHD caused by dominant mutations in *GHRHR* with variable penetrance.

Other Genetic Factors and IGHD

The growth hormone secretagogue receptor (*GHSR*, OMIM 601898) is expressed in the hypothalamo-pituitary area and its endogenous ligand, ghrelin, has a role in the regulation of the release of GH [261, 262]. Recessive and

dominant *GHSR* mutations (p.W2X, p.R237W, p.A204E) have been reported to result in phenotypes that range from normal to partial IGHD with a mechanism of action that is not fully elucidated but may be associated with loss of the constitutive activity of the receptor [263, 264].

Severe IGHD and anterior pituitary hypoplasia have been reported in patients with biallelic *RNPC3* (OMIM 618016) mutations [265]. The gene codes for a minor spliceosome protein. In this case, three sisters from a non-consanguineous pedigree were born with normal length and weight and showed severe postnatal proportionate growth retardation (height –5 to –6.6 SDS), typical physical features of GH deficiency, delayed bone maturation without bone dysplasia and mild microcephaly. They had undetectable GH after stimulation, undetectable IGF-1 and IGF-BP3 and low-normal PRL concentrations. Pituitary MRI confirmed anterior pituitary hypoplasia.

Congenital Central Hypothyroidism

Central hypothyroidism has a reported prevalence of 1 in 50,000 live births [266, 267]. It is characterized by insufficient TSH secretion and low concentrations of thyroid hormones. Familial cases have been reported, although the condition may also be sporadic. Central hypothyroidism is generally milder than primary hypothyroidism and neonates may present with non-specific symptoms such as lethargy, poor feeding, failure to thrive, prolonged hyperbilirubinaemia and cold intolerance.

Congenital isolated TSH deficiency may result from homozygous mutations of the *TSHβ* gene (OMIM 188540) or inactivating mutations in the TRH receptor gene (OMIM 188545). Patients present with absence of TSH due to *TSHβ* mutations [268, 269] and, in the case of TRH receptor mutations, impaired TSH and PRL responses to TRH [270, 271].

Mutations in the immunoglobulin superfamily member 1 (*IGSF1*, OMIM 300137) are the most recently identified cause of central hypothyroidism, with an estimated incidence of up to 1 in 100,000 [272, 273]. *IGSF1* is located on the X chromosome and encodes a membrane glycoprotein expressed in Rathke's pouch and in the adult pituitary gland [274]. Loss-of-function mutations in *IGSF1* have been reported in males with central hypothyroidism, either isolated or associated with hypoprolactinaemia [272, 274–276]. A minority of patients require treatment for transient partial GH deficiency in childhood [272]. There is a delayed pubertal growth spurt but all affected individuals develop macroorchidism in adulthood [273, 274]. IGSF1 protein is detected in thyrotrophs, somatotrophs and lactotrophs but not gonadotrophs [274]. In the rat, pituitary expression is also confined to cells of the *Pou1f1* lineage. Additionally, up to 33% of carrier females manifest a phenotype characterized by TSH deficiency [272].

Isolated ACTH Deficiency

Congenital isolated ACTH deficiency is rare; ACTH deficiency is more commonly associated with other pituitary hormone deficiencies. The clinical features are poorly defined and patients usually present in the neonatal period with non-specific symptoms (poor feeding, failure to thrive, hypoglycaemia) or more acute signs of adrenal insufficiency (vascular collapse, shock). Abnormalities in salt excretion are unusual because aldosterone secretion is largely controlled by the renin–angiotensin system.

A few cases of isolated ACTH deficiency have been reported due to mutations in *POMC* (OMIM 176830) and *TBX19* (*T-PIT*, OMIM 604614). Patients with homozygous or compound heterozygous mutations in *POMC* present with early-onset isolated ACTH deficiency, obesity and red hair due to a lack of MSH production [277, 278]. Recessive *T-PIT* mutations are the main molecular cause of congenital neonatal isolated ACTH deficiency, which tends to be severe resulting in profound hypoglycaemia associated with seizures and prolonged cholestatic jaundice [21, 279–282]. Neonatal deaths have been reported in up to 25% of families with *T-PIT* mutations, suggesting that isolated ACTH deficiency may be an underestimated cause of neonatal death [281]. Patients present with very low basal plasma concentrations of ACTH and cortisol with no significant ACTH response to CRH.

Prohormone convertase 1 (PCSK1, OMIM 162150) mutations are rare and lead to ACTH deficiency in association with hypogonadotropic hypogonadism and a complex phenotype, including a child with isolated ACTH deficiency, red hair and severe enteropathy [283]. A compound heterozygous PCSK1 mutation has also been described in a female patient with extreme early-onset obesity and ACTH deficiency, hypogonadotropic hypogonadism, defective processing of other prohormones and insulin-dependent diabetes mellitus [284].

Central Diabetes Insipidus (DI)

Congenital central DI is rare and may be a feature of midline disorders (SOD, HPE) or due to mutations in genes involved in the secretion of AVP. A number of mutations have been described in the gene that encodes an AVP preprohormone, AVP–neurophysin II (*AVP-NPII*, OMIM 192340), resulting in autosomal dominant central DI [285–288]. The gene is located on chromosome 20 and consists of three exons [289, 290]. Exon 1 encodes the signal peptide of the preprohormone and AVP, exon 3 encodes the glycoprotein copeptin, while the carrier protein NPII is encoded by all three exons.

In this rare familial disorder of AVP secretion, patients usually present in the first 10 years of life but neonatal manifestations are uncommon, which suggests that the pathophysiology of familial central DI involves progressive postnatal degeneration of AVP-producing

magnocellular neurons. The proposed mechanism is that the mutant allele exerts a dominant negative effect; the misfolded mutant hormone precursor is accumulated in the endoplasmic reticulum resulting in progressive damage to the AVP neurons and the eventual clinical manifestation of DI (see also Chapter 14, 'Disorders of Water Balance') [291, 292].

Central DI is a feature of Wolfram syndrome, a rare recessive disorder characterized additionally by diabetes mellitus, optic atrophy, sensorineural deafness and progressive neurodegeneration (DIDMOAD syndrome). The gene *WFS1* (OMIM 606201) is located on 4p16.1 and encodes wolframin [293]. The protein is localized in the endoplasmic reticulum and is a component of the misfolded protein/stress response mechanism [294]. *WFS1* expression has been detected in selected neurons in the hippocampus, amygdala and olfactory tubercle [295].

Acquired Disorders of Hypothalamo-Pituitary Dysfunction

While research on the various congenital forms of hypothalamo-pituitary dysfunction in rodent models and human genetic studies has revealed the intricacies of the pathways involved in pituitary development, congenital forms of hypopituitarism are rare and account for only 4.5% of hypopituitarism in adults [296]. The close proximity of the hypothalamo-pituitary axis to various vital structures means that acquired damage to this area can easily lead to hypopituitarism.

In adult cohort studies, suprasellar tumours cause the majority of hypothalamo-pituitary dysfunction (50–60%) [1, 296]. Other causes include surgery or radiotherapy, traumatic brain injury (TBI), infection, autoimmune processes, infiltration by granulomatous disease, iron overload states and vascular causes (Tables 5.10 and 5.11). The effect of oncological treatments such as surgery and radiotherapy on the hypothalamo-pituitary axis is presented in Chapter 13 ('Endocrine Late Effects of Cancer Treatment').

Table 5.10 Common acquired causes of hypothalamo-pituitary dysfunction.

Suprasellar tumours
Neoplastic
Craniopharyngioma
Low-grade optic pathway glioma (mainly pilocytic astrocytoma)
Germ cell tumours (mainly germinoma)
Pituitary adenoma
Hamartoma
Chordoma
Meningioma
Metastases (Hodgkin's lymphoma, nasopharyngeal carcinoma)
Non-neoplastic
Rathke's cleft cyst
Arachnoid cyst
Epidermoid/dermoid cysts
Radiotherapy
Radiotherapy for CNS tumours
Radiotherapy for haematological malignancies and bone marrow transplant
Brain trauma
Traumatic brain injury (accidental and non-accidental)
Neurosurgery
Inflammation/infection
Meningitis/encephalitis
Pituitary abscess
Sarcoidosis
Tuberculosis
Autoimmune (lymphocytic hypophysitis)
Infiltration
Langerhans cell histiocytosis
Iron overload (hereditary haemochromatosis, secondary haemochromatosis from repeated transfusions e.g. thalassaemia)
Psychosocial deprivation

Central Nervous System (CNS) Tumours

CNS tumours are the commonest childhood malignancy after leukaemias, accounting for 25% of cancers in children <15 years of age with an annual incidence rate of 35 cases/million/year [301–304]. As with all childhood cancers, the incidence is increasing worldwide [301, 302, 305], mainly due to improvements in diagnosis and tumour registration [306–308]. Campaigns such as the HeadSmart project have been aimed at increasing awareness of pediatric brain tumour symptoms and therefore earlier diagnosis (http://www.headsmart.org.uk/) [309].

Five-year survival for CNS tumours has increased from 57 to 65% in the last decade (~95% in low-grade gliomas) due to improved multimodality cancer therapies and better supportive care [310–312]. High survival rates due to increasingly intensive treatment strategies aimed at improving cure in a small minority of cases can cause a higher toxicity burden in the larger majority and a rapidly expanding cohort of survivors is faced with reduced quality of life due to late and evolving multiorgan toxicities [313–315]. More than 80% of CNS tumour survivors develop at least one endocrinopathy, most frequently GH deficiency [316].

Tumour location and histology are age dependent with 5 and 16% of CNS tumours diagnosed in childhood (<14 years) and young adulthood (15–24 years) being suprasellar [317]. 10% of all pediatric CNS tumours are supra- or intrasellar [317, 318] and can be benign or malignant, cystic or solid. In children, these tumours are largely primary lesions; secondary metastases to this region from distant organs are very rare.

Table 5.11 The differential diagnosis of pediatric suprasellar masses by neuroradiological features.

Lesion	Primary location	T_1-weighted appearances	T_2-weighted appearances	Contrast enhancement	Other features
Craniopharyngioma	Supra > intrasellar	Variable, heterogeneous	Hyperintense	Yes (cystic rims)	Cysts, calcification
Low-grade glioma	Suprasellar, optic pathways	Hypointense	Hyperintense	Yes	Generally homogeneous
Pituitary adenoma	Intrasellar	Hypointense	Hyperintense	No	Sella turcica expansion
Germinoma[a]	Suprasellar, infundibulum	Iso-hypointense	Iso-hypointense	Yes	Loss of posterior pituitary bright spot, coexistent pineal tumour
Hamartoma	Suprasellar (tuber cinereum)	Isointense	Iso-hyperintense	No	—
Langerhans cell histiocytosis[a]	Suprasellar, infundibulum	Isointense	Isointense	Yes	Loss of posterior pituitary bright spot, coexistent osseous lesions
Lymphocytic hypophysitis[a]	Suprasellar, infundibulum, intrasellar	Isointense	Isointense	Yes	Loss of posterior pituitary bright spot
Granuloma[a] (sarcoidosis, TB)	Suprasellar, infundibulum	Iso-hypointense	Iso-hypointense	Yes	Coexistent parenchymal and leptomeningeal lesions
Rathke's cleft cyst	Intrasellar	Iso-hyperintense	Iso-hypointense	No	Round and smooth walled
Arachnoid cyst	Suprasellar	Hypointense (isointense with CSF)	Hyperintense (isointense with CSF)	No	—

[a] Note that germinomas, LCH, lymphocytic hypophysitis and granulomatous lesions are not easily differentiated on radiological features alone [297–300].

The close proximity to the hypothalamo-pituitary axis means that survivors are at increased risk of endocrine dysfunction secondary to tumour mass effect and/or treatment manifesting either at presentation or evolving over several decades. The close relationship with other vital structures such as the ventricles, optic nerves and chiasm also means that the vast majority of patients present with symptoms or signs of raised intracranial pressure (ICP) or visual disturbances [319]. Tumours can additionally cause clinically evident endocrine symptoms and signs by hormone hypersecretion, although this is less common in childhood. Hypothalamic damage also leads to non-endocrine sequelae such as temperature dysregulation, hyperphagia, obesity and sleep disorders, the pathophysiology of which is still largely unknown.

Craniopharyngiomas

Epidemiology and Histology

Craniopharyngiomas are benign non-gliomatous CNS tumours (World Health Organization [WHO] classification grade I [320]) originating from embryological remnants of the Rathke's pouch [321]. Although rare (incidence 1.1–1.7 cases/million/year [322–324]), they are the commonest suprasellar tumour in childhood, accounting for up to 80% of tumours in this region [325, 326] and contributing to 1.5–11.6% of all pediatric CNS tumours [297, 298, 322, 327, 328]. There is a well-recognized bimodal distribution in age-specific incidence, with peaks between 5–14 and 65–74 years of age [322, 323]. Thus, the incidence of craniopharyngiomas in the pediatric population is slightly higher, with a recent epidemiological study estimating a WHO-standardized age-specific incidence of 2.1 cases/million/year in children <15 years [323]. Craniopharyngiomas can, however, be diagnosed at any age, with cases being reported in the neonatal period [329–331].

Histologically, childhood craniopharyngiomas are almost invariably adamantinomatous, referring to calcifications in a significant proportion and their similarity to adamantinomas, a rare bone cancer [332, 333]. The papillary subtype is much rarer and found almost exclusively in adults. In a pediatric subcohort of craniopharyngiomas undergoing surgery, 56.7% were mainly cystic, 16.7% had multiple cysts, 13.3% were mainly solid, 10.0% were entirely solid, and 3.3% were entirely cystic [334]. The cystic fluid is viscous and rich in cholesterol crystals, producing what is described as an "engine oil" consistency. Despite the benign histology, the invasive nature and location close to vital hypothalamo-pituitary structures makes craniopharyngiomas the archetypal suprasellar tumour for causing

neuroendocrine dysfunction from both tumour- and treatment-related effects.

Genetics

Craniopharyngiomas are typically sporadic, and only two case reports of familial craniopharyngiomas exist in published English literature, one of these occurring in siblings from a consanguineous pedigree [335] while the second occurred in a mother and her daughter, although the latter were of the papillary subtype [336]. Craniopharyngiomas have occurred in association with other CNS tumours such as glioblastoma multiforme [337] and Turner [338], Russell–Silver [339], Kallmann [340], Aicardi [341], Duane [342], Gardner [343, 344] and Bardet–Biedl syndromes [345]. Multiple chromosomal abnormalities have been reported in a few cases but these are not always consistent [346–348].

Mutations in the *PTCH1* (patched homologue 1) tumour suppressor gene that is usually associated with the Gorlin syndrome (an oncogenic disorder associated with naevoid basal cell carcinomas, keratocytic odontogenic jaw tumours, medulloblastomas, ovarian fibromas, cardiac fibromas and other CNS tumours [349]) have been found to result in loss of heterozygosity in craniopharyngiomas and an increased expression of β-catenin [350]. Detailed examination of such mutations in both human and mouse models has revealed the role of increased activation of the *SHH* and Wnt/β-catenin pathways in craniopharyngioma tumorigenesis [351, 352]. SHH is the ligand for PTCH1 and binding to its receptor causes the accumulation of SMO (smoothened), which in turn increases cell proliferation. Similarly, Wnt binds to the Frizzled receptor and prevents the degradation of β-catenin (*CTNNB1*), a cytoplasmic protein that accumulates and acts as a transcriptional co-activator and mediates cell–cell adhesion. Indeed mutational screens of human adamantinomatous craniopharyngioma samples indicate a high prevalence of mutations in *CTNNB1* that are compatible with abnormal accumulation of the protein [353–356]. β-Catenin accumulation occurs in cell clusters that overexpress SHH and are the histopathological landmark for adamantinomatous craniopharyngioma [351, 357, 358]. More recent experiments have demonstrated that expression of mutant β-catenin results in tumorigenesis only in Rathke's pouch embryonic progenitors and not in differentiated cells, emphasizing the embryonic origin of these tumours [22, 352].

Clinical Presentation

Like many other suprasellar tumours, the time to diagnosis from initial onset of symptoms is often significantly delayed, with a median symptom duration of 8 months to 8 years due to their location deep within the brain parenchyma (Table 5.12) [359–361, 365, 370, 371]. The commonest presenting symptoms in childhood relate to raised ICP, with 51–78% of patients experiencing headaches and 31–61% having nausea and/or vomiting [359–364], all of which are more frequent in children compared with adults [360]. Visual deterioration is the second commonest symptom, presenting either as a reduction in visual acuity (23–84%) or as a restriction in visual fields (17–57%); given the typical age at presentation, this may not be recognized until severe [359–362, 364–366]. Papilloedema is observed in 29% of patients [360, 363]. Neurocognitive symptoms are less common but include cranial nerve palsies, ataxia, hemiparesis, seizures, cognitive impairment and behavioural changes.

Symptoms related to hypothalamo-pituitary dysfunction are under-recognized but are the third commonest group at diagnosis. Muller et al. [318] demonstrated increase in BMI and linear growth failure from as early as 6 to 7 months and 10 to 12 months of age, respectively, before the diagnosis of craniopharyngioma. DeVile et al. [367] showed a marked discrepancy between the proportion of patients who reported endocrine-related symptoms, such as linear growth retardation, weight loss or gain and polyuria and polydipsia as the initial presenting complaint, and those who had these manifestations on direct enquiry or examination.

Linear growth failure is the commonest endocrine-related sign at diagnosis (14–86%) [359–363, 365, 367, 368], in keeping with GH deficiency being the most frequent endocrine deficit. Central DI is particularly likely to go undiagnosed until the patient is water deprived or rendered effectively adipsic by general anaesthesia, coma or further hypothalamic damage sustained perioperatively, with potentially fatal neurological sequelae. Non-specific symptoms such as lethargy, prolonged recovery from recurrent infections, somnolence and cold intolerance may be related to ACTH and/or TSH deficiencies. Craniopharyngiomas often present with pubertal delay or arrest in adolescents [362] but precocious puberty can also occur [372]. Hypothalamic dysfunction may present as hyperphagia, escalating obesity, sleep–wake cycle disturbances and temperature dysregulation. Patients presenting with any of these features warrant endocrine referral and neuroimaging.

Investigations

Radiology The coexistence of solid, cystic and/or calcified structures on neuroimaging is often sufficient to make a diagnosis without biopsy (Figure 5.8). The cystic rim of the lesion is usually hyperintense on T_1- and T_2-weighted MRI sequences and demonstrates contrast enhancement. The degree of intensity of the cyst contents depends on the proportion of protein and free methaemoglobin [333, 373]. The solid component of the tumour is usually hyperintense on T_2-weighted imaging and enhances heterogeneously [373]. Between

Table 5.12 Common symptoms and signs at presentation of craniopharyngioma ranked by median frequency.

Presenting symptom/sign	Median frequency (range)
Headaches [359–364]	64% (51–78)
Reduction in visual acuity [359–366]	51% (23–73)
Restriction in visual fields [359–365]	46% (17–61)
Nausea/vomiting [359–364]	43% (31–61)
Linear growth failure/short stature [359–363, 365, 367–369]	**33% (14–86)**
Papilloedema [363]	29%
Lethargy/somnolence [359, 360, 367]	**21% (5–22)**
Cranial nerve palsy [359, 360, 363]	20% (11–27)
Weight loss [359, 360, 365, 367]	**17% (5–31)**
Polyuria/polydipsia [359, 360, 362, 363, 365, 367]	**16% (9–28)**
Pubertal delay/arrest [359, 360, 362, 363, 367]	**10% (5–24)**
Cognitive impairment [360]	10%
Blindness [360, 365]	9% (3–15)
Ataxia [321, 359, 363]	8% (7–18)
Hemiparesis [321, 359, 363, 365]	8% (7–12)
Decreased consciousness [360, 363]	8% (5–10)
Hyperphagia/weight gain [359, 360, 365, 367]	**6% (5–30)**
Seizures [359, 363, 365]	5% (5–6)
Optic atrophy [360]	5%
Behaviour change/psychiatric symptoms [359, 360, 365]	4% (3–10)
Gynaecomastia/galactorrhoea [359]	**4%**
Cold intolerance [359, 360]	**3% (0–5)**
Precocious puberty [362, 363, 365, 367]	**2% (0–3)**
Sleep/wake cycle disturbance [359]	**2%**

Symptoms in bold indicate probable underlying hypothalamo-pituitary dysfunction, which may not be recognized.

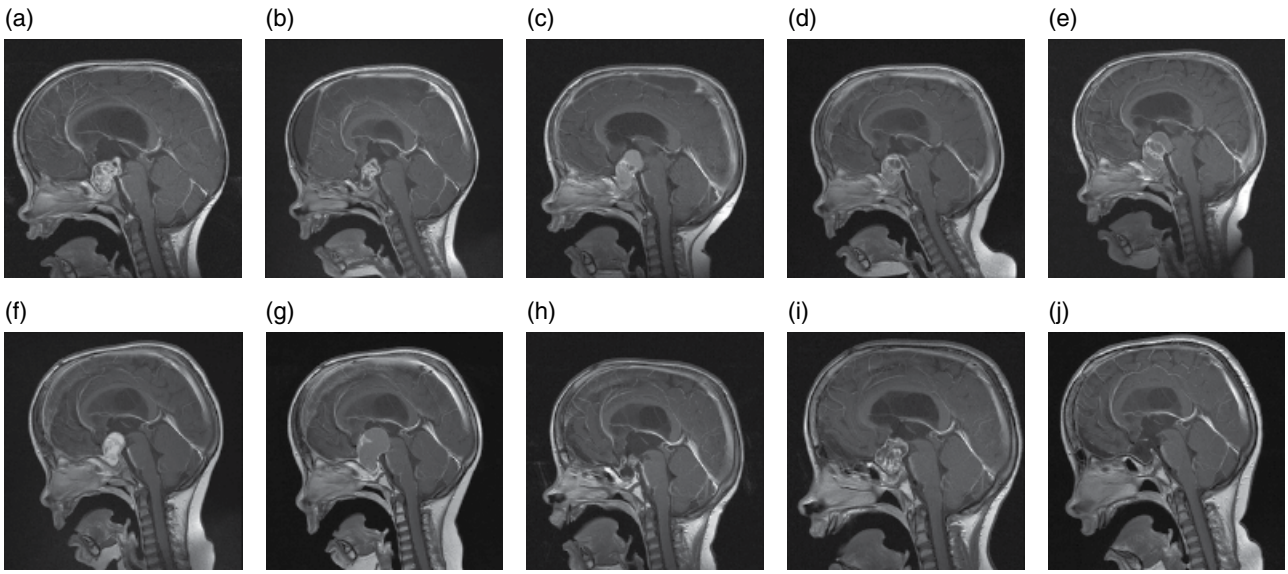

Figure 5.8 Serial T_1-weighted MRI images with gadolinium contrast of a patient who presented at age of 2 years with a craniopharyngioma demonstrating solid, cystic and calcified components and the tendency for multiple progressions over 7 years. (a) After initial endoscopic cyst fenestration and ventriculoperitoneal shunt insertion, (b) after first transcranial debulking, (c) first cystic progression, (d) after first cyst drainage via reservoir, (e) second cystic progression, (f) after second transcranial debulking, (g) after adjuvant radiotherapy and third cystic progression, (h) after second cyst drainage via reservoir, (i) after fourth cystic and solid progression, (j) after complete resection.

65 and 93% of these tumours contain calcified components [332, 333].

Seventy-five percent of craniopharyngiomas are suprasellar with an intrasellar extension, 20% are exclusively suprasellar and 5% are exclusively intrasellar. Over 50% involve the hypothalamus and almost one-third invade the floor of the third ventricle, potentially causing obstructive hydrocephalus [298, 360, 366]. Several radiological grading systems have been proposed to describe hypothalamic integrity and the risk of tumour- and treatment-related long-term morbidity to aid decision-making and surgical planning but no consensus has been reached as to which is best [365, 374–376].

Endocrinology Depending on the cohort examined, 46–100% of patients have hypothalamo-pituitary endocrine dysfunction at diagnosis, and up to 20% have panhypopituitarism [359, 363]. As with most other suprasellar tumours, GH deficiency is the commonest endocrinopathy at presentation (35–100%), depending on whether dynamic tests were performed on all patients or only on those presenting with growth failure [359, 360, 363, 367, 368, 377]. Eighty percent have low IGF-1 concentrations [362, 369, 378].

This is followed by LH/FSH (10–85% based on biochemical testing; 5–24% have pubertal delay/arrest), ACTH (21–71%), TSH (13–32%), PRL (8–32%) and ADH (17–29%) deficiencies [359, 360, 362, 363, 367, 369, 377]. Hyperprolactinaemia due to pituitary stalk compression occurs in 11–52% but is usually asymptomatic [359, 362, 367]. Precocious puberty is a presenting feature in 3% of patients [362, 363, 365].

Baseline pituitary function testing before treatment is recommended for all patients, with additional dynamic function testing as clinically indicated. Life-threatening endocrine deficits such as ACTH deficiency and central DI must be excluded before surgery, the former particularly if dexamethasone is not started for peritumoral oedema.

Ophthalmology and Neuropsychology Given the prevalence of neuro-ophthalmological symptoms and signs at presentation, specialist referrals should be made for baseline neurology, visual acuity, visual fields and colour vision assessments. Visual evoked potentials (VEPs) are increasingly used to assess children unable to cooperate with traditional visual acuity testing. Neuropsychological testing by specialists used to dealing with visually impaired children should also be performed as early as possible in the management pathway to provide a baseline for assessing future post-treatment morbidity.

Treatment and Outcome

The management of craniopharyngioma is complex and best achieved through a multidisciplinary approach in a specialist unit. Previous UK consensus risk-based treatment strategy guidelines [379] are in the midst of being updated under the auspices of the National Institute for Health and Care Excellence, the Royal College of Paediatrics and Child Health, the British Society for Paediatric Endocrinology and Diabetes and the UK Children's Cancer and Leukaemia Group.

Treatment should aim to relieve symptoms of raised ICP, preserve vision and minimize hypothalamo-pituitary dysfunction while providing long-term control and reduced recurrence rates [379, 380]. Hypothalamic invasion indicates a high risk of acute and long-term morbidity and a less aggressive surgical approach to avoid irreversible, potentially fatal hypothalamic damage is recommended. Risk factors for poor prognosis include age <5 years, tumour height >3.5–5 cm in the midline, severe hydrocephalus and tumour adherence to surrounding healthy tissue at surgery [334, 365, 374, 375, 381, 382].

Complete tumour resection increases the likelihood of overall and progression-free survival (PFS) compared with subtotal resection (10-year PFS 73–100% vs. 28–53%) [334, 359, 360, 371, 374, 383] but some of these studies are specialty biased and suffer from insufficient follow-up, which does not assess the long-term hypothalamo-pituitary morbidity found by others [334, 361, 363, 365, 374, 384–386]. Some authors advocate adjuvant radiotherapy after incomplete resection because this has been shown to achieve similar PFS rates to complete resection alone with potentially less hypothalamo-pituitary dysfunction (5-year PFS 73–82%) [360, 387, 388]. The optimum timing of radiotherapy after resection or only on progression has still to be determined and is the subject of a multicentre randomized German trial (KRANIOPHARYNGEOM 2007) [383]. Newer techniques, such as proton beam therapy, are being explored and there is little experience in children with radiosurgery or intracystic therapies such as interferon-α.

Post-operative hypothalamo-pituitary endocrine dysfunction is extremely common and the perioperative period must be closely supervised due to the risk of the well-recognized triphasic phenomenon of posterior pituitary dysfunction: transient central DI in the first 48 hours can be followed by inappropriate ADH secretion lasting up to 2 weeks culminating in a second phase of central DI, which can be permanent [389–391]. Because of the possibilities of coexisting ACTH deficiency, cerebral salt-wasting syndrome, hypothalamic adipsia from surgery and concurrent use of ADH-inducing anticonvulsants, fluid and electrolyte imbalances are frequent and may be very difficult to control (Figure 5.9).

Ninety-seven percent of survivors of treatment have at least one hypothalamo-pituitary deficit and 31–84% have panhypopituitarism [359, 361, 363, 364, 367, 371, 374, 382, 386, 392–394]. Endocrine deficits evolve and

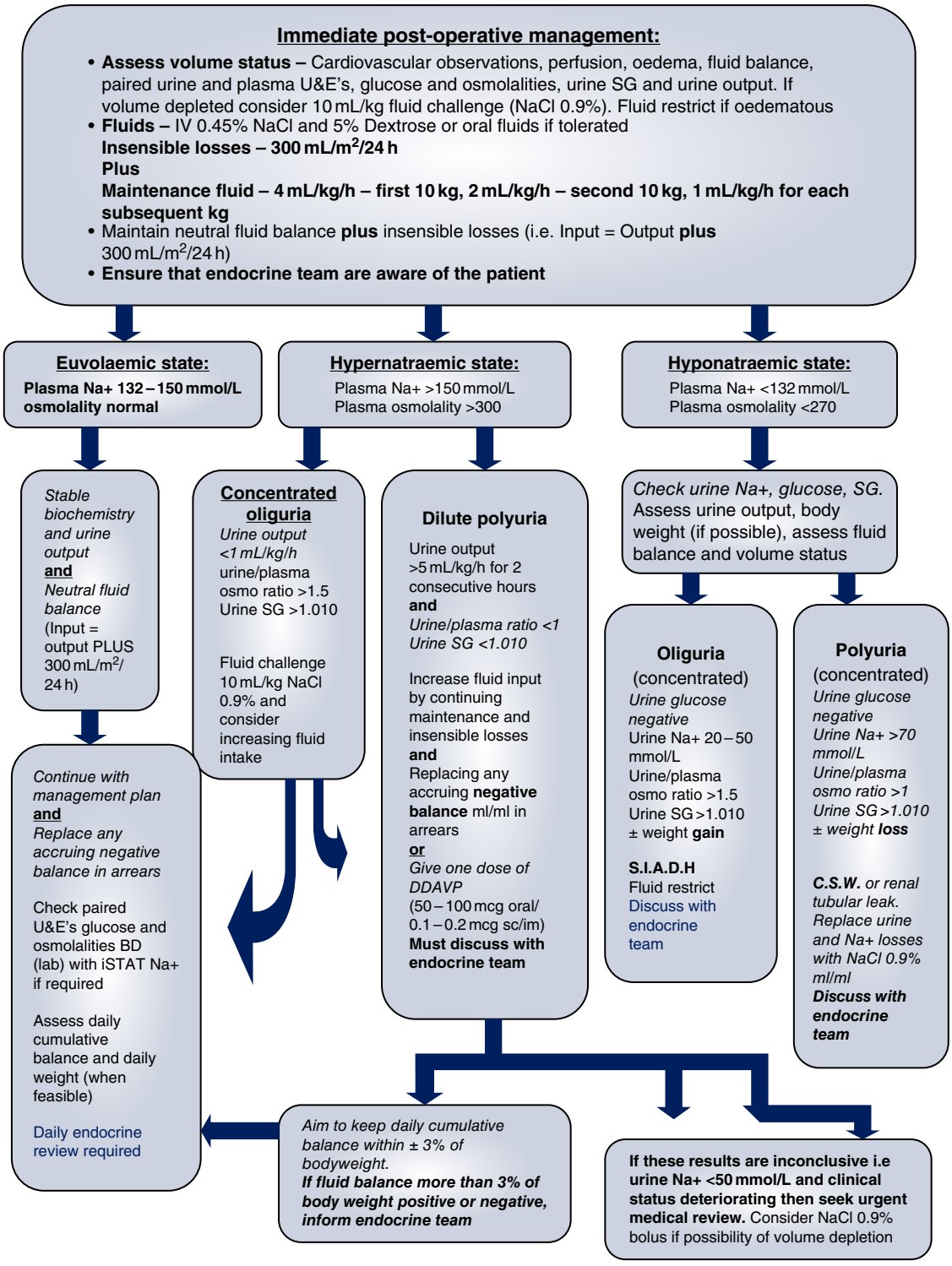

Figure 5.9 Suggested algorithm for differentiating between central DI, cerebral salt wasting and the syndrome of inappropriate ADH secretion (SIADH).

follow the hierarchical pattern observed in other suprasellar tumours and preoperatively: GH deficiency is commonest (20–99%), followed by LH/FSH (30–95%), TSH (39–97%), ACTH (39–89%) and ADH (42–94%) deficiencies [359–361, 363, 364, 367, 370, 371, 382, 386, 392–395].

GH replacement in craniopharyngioma patients does not increase the risk of progression or of second tumours [383, 396]. Hypothalamic obesity or hypothalamic syndrome is a frequent yet poorly understood and therefore untreatable complication for up to two-thirds of craniopharyngioma survivors, who fulfil many of the risk factors for this complication [385]. Consequently all patients should be followed carefully with clear transition plans between pediatric and adult services to monitor for the lifelong risk of further endocrine deficits and the cardiometabolic risks of obesity.

Low-Grade Gliomas

Epidemiology and Histology

Low-grade gliomas (LGGs) are benign grade I or II astrocytic tumours [320], which comprise more than 40% of childhood CNS tumours [304, 308]. There is a bimodal age distribution in incidence with peaks between 3–5 and 13–15 years [308, 312]. The majority of these tumours are juvenile pilocytic astrocytomas, although other histological subtypes are recognized, including diffuse fibrillary, pilomyxoid and subependymal giant cell astrocytomas. 30–50% of these tumours affect the optic pathway, hypothalamus and suprasellar midline, making LGGs the second commonest tumour of this region after craniopharyngiomas [312, 397]. Suprasellar LGGs exhibit the same unpredictable growth patterns despite their benign histology and can also undergo spontaneous involution and late-onset progression and/or result in leptomeningeal metastases [398–401]. Their potential for causing long-term tumour- and treatment-related neuroendocrine morbidity is significant.

Genetics

10–16% of LGGs are associated with neurofibromatosis type 1 (NF-1), an autosomal dominant disorder caused by a deactivating mutation in the *NF1* (neurofibromin 1) tumour suppressor gene. Loss of function of neurofibromin in LGGs results in over-activation of the Ras oncogenic pathway and tumorigenesis [402]. The relationship with NF-1 is strongly dependent on tumour location; 27–40% of hypothalamochiasmatic and 70% of isolated optic nerve LGGs are NF-1 associated [311, 312, 397, 403]. LGGs in patients with NF-1 tend to be more asymptomatic (15% of NF-1 patients have asymptomatic LGGs on MRI screening [404]) and behave more indolently, despite occurring more anteriorly, bilaterally and multifocally within the optic pathway [312, 401, 405–407].

Molecular genetic studies of pediatric-specific pilocytic astrocytoma mutations indicate that a significant proportion show changes resulting in fusion between the proto-oncogenes *KIAA1549* and *BRAF*, resulting in activation of the mitogen-activated protein kinase (MAPK) pathway, a necessary step in tumorigenesis of many cancers [408]. These mutations have been the focus for development of novel molecular therapies [409] but none is yet in clinical use.

Clinical Presentation

The commonest symptom at diagnosis is visual impairment (50–100%), followed by raised ICP (22–86%) [398, 410–412]. Ophthalmological symptoms such as strabismus (15–38%), nystagmus (11–38%) and proptosis (3–38%) are also relatively common, as are sensorimotor dysfunction, seizures and developmental or behavioural changes [400, 405, 406, 410, 413–415]. Endocrine dysfunction has been thought to be uncommon at presentation but wide variations in reported incidence at diagnosis have been reported, with up to 59% of patients having symptoms and signs attributable to endocrinopathy [406, 413, 416, 417].

In contradistinction to craniopharyngiomas, disorders of the gonadotropin (LH/FSH) axis seem most prevalent at presentation, with central precocious puberty being particularly common but also pubertal delay/arrest [416]. Hypothalamic LGGs can present in infancy with the diencephalic syndrome, which comprises severe emaciation with a normal or accelerated height velocity, GH excess, hyperactivity and euphoria, the pathophysiology of which is unknown [311, 417–419].

Investigations

Radiology Suprasellar LGGs in children appear as well-demarcated T_1-hypointense and T_2-hyperintense tumours on MRI, with varying degrees of cystic components and contrast enhancement [401, 420]. Unlike craniopharyngiomas, calcification is extremely rare [298]. The frequency of direct involvement of the optic pathway means that histological diagnosis is not always necessary, particularly in the context of NF-1. An anatomical classification system for staging optic pathway LGGs was first proposed in 1958 [209] but this has been modified to help distinguish bilateral, asymmetrical and multifocal tumours as well as differentiate between the involvement of the optic tracts posteriorly, the hypothalamus superiorly and the leptomeninges distantly [407].

Endocrinology The incidence of hypothalamo-pituitary endocrine dysfunction at diagnosis is variable. Central precocious puberty occurs in up to 56% of patients at diagnosis, followed by deficiencies in GH (up to 27%), LH/FSH (up to 12%), TSH (up to 10%) and ACTH (up to 3%) [413, 414, 416, 421]. Central DI occurs in just over 10% of patients [416, 422]. In the diencephalic syndrome, GH excess is often accompanied by a normal or low IGF-1 concentration [418, 419] and increased resting energy expenditure [423, 424]. Other less common endocrinopathies have included acromegaly [425] and SIADH [426].

Ophthalmology and Neuropsychology Visual and cognitive function should be documented at diagnosis. Radiological appearances neither correlate with the degree of visual disturbance observed nor predict post-treatment visual prognosis [398, 427–429]. VEPs help assist in determining visual function and guide decision-making in younger children.

Treatment and Outcome

Complete tumour resection has long been shown to be a favourable risk factor for survival [312, 397] but this is rarely possible without leading to major visual and neuroendocrine morbidity with suprasellar and/or optic pathway tumours. Thus, while actuarial 5-year overall survival (OS) is high (up to 95%), this is mitigated by a significantly reduced PFS (34–69%) with a potential for late progression and mortality [311, 312, 397, 416, 430–432]. Treatment trials have focused on medical strategies for managing these tumours, with radiotherapy being largely delayed in favour of chemotherapy (usually involving carboplatin, vincristine, cyclophosphamide and/or cisplatin) in young children because of concerns regarding cognitive dysfunction [433], subsequent adjuvant cancers [434] and radiation-induced vasculopathies [435]. To date none of the international multimodality LGG trials – LGG1 (1997–2004) and LGG2 (2005–2010) – have been randomized, these being observational studies aimed at improving visual outcomes with little reported success [311, 312]. LGG3 is now being designed with careful long-term prospective measurements of visual and neuroendocrine outcomes.

A 30-year analysis by Gan et al. [416] has indicated that long-term endocrine event-free survival (EEFS) is significantly reduced compared with OS or PFS, with four of five survivors having hypothalamo-pituitary endocrine dysfunction at 20 years. In this multivariate analysis, hypothalamic tumour location predicted the earlier onset of endocrinopathy rather than radiotherapy exposure, which predicted its density (the total number of hypothalamo-pituitary axes affected). There was a suggestion that some endocrinopathies, e.g. GH deficiency, were becoming more frequent with successive treatment eras and posterior pituitary dysfunction occurred even in the absence of major tumour resection (e.g. procedures involving tumour biopsy or shunt insertions).

The incidence of dysfunction mimics that of craniopharyngiomas, with GH deficiency being commonest, followed by central precocious puberty and LH/FSH deficiency and finally by deficiencies in TSH, ACTH and central DI [397, 416]. Hypothalamic obesity is relatively common and faced by nearly a third to a half of survivors [397, 416], so lifelong follow-up is essential, as for craniopharyngiomas.

Pituitary Adenomas

Epidemiology and Histology

Unlike adults, pituitary adenomas are rare in childhood and account for <3% of all supratentorial tumours with an annual incidence of 0.1 cases/million/year [436, 437]. This means that they are often not considered in the differential diagnosis of suprasellar tumours in this age group but the difference in treatment strategy from craniopharyngiomas or LGGs highlights the importance of routine endocrine evaluation in all such cases. Non-functioning pituitary adenomas are unusual [438, 439] and the majority of pituitary adenomas are hormonally active, arising from any of the five cell types of the anterior pituitary, secreting their corresponding hormones and resulting in symptoms and signs of hormone excess.

Prolactinomas (PRL, 53%) are commonest, followed by corticotrophinomas (ACTH, 35%), somatotrophinomas (GH, 9%) and, much less commonly, thyrotrophinomas (TSH) or gonadotrophinomas (LH/FSH) [440]. The distribution of tumour subtype is age dependent, prolactinomas being commonest in adolescence and corticotrophinomas commonest in the first decade of life [439, 440]. All pituitary adenomas tend to appear as hypointense, poorly enhancing lesions on T_1- and T_2-weighted MRI sequences.

Genetics

9–22% of pediatric pituitary adenomas are associated with genetic tumour-predisposing syndromes, the best known of which is multiple endocrine neoplasia type 1 (MEN-1), with a prevalence of 0.02–0.2 cases per 1000 [441, 442]. MEN-1 is an autosomal dominant condition characterized by parathyroid adenomas (leading to primary hyperparathyroidism), pituitary adenomas, enteropancreatic tumours, non-functioning adrenocortical adenomas, angiofibromas, collagenomas, thyroid adenomas, meningiomas and other neuroendocrine tumours [443]. Primary hyperparathyroidism is the commonest presenting manifestation of MEN-1 but 17% of patients have a pituitary adenoma, usually a prolactinoma [444]. Other genetic syndromes (and their associated genes)

associated with pituitary adenomas are the familial isolated pituitary adenoma (FIPA) syndrome (*AIP*), multiple endocrine neoplasia type 4 (*CDKN1B*), Carney complex (*PRKAR1A*), McCune–Albright syndrome (*GNAS*), SDH-related pituitary adenoma syndrome (*SDHB*, *SDHC*, *SDHD*) and *DICER1* syndrome [445, 446]. Corticotrophinomas specifically demonstrate an increased frequency in somatic *USP8* mutations, which is also associated with an increased risk of recurrence [447]. Many of these mutations result in either oncogene activation or tumour suppressor inactivation. Given their rarity and therefore the possibility that a child presenting with a pituitary adenoma may be the index case for a familial tumour predisposition syndrome, some authors advocate testing for the commonest two mutations found in pituitary adenomas – *MEN1* (mainly prolactinomas) and *AIP* (mainly somatotrophinomas) – with an algorithm for testing being suggested by Korbonits et al. [446] (Figure 5.10).

Prolactinomas

Prolactinomas account for 50–70% of all pituitary adenomas in children [439, 448, 449] and are classified into microprolactinomas (<1 cm), macroprolactinomas (>1 cm) and giant prolactinomas (>4 cm) with serum PRL increasing with tumour size. The differential diagnosis of hyperprolactinaemia includes stalk compression by tumour mass (interrupting dopaminergic inhibition of PRL secretion), drugs (e.g. phenothiazines, metoclopramide) and the presence of macroprolactin (PRL bound to IgG) but concentrations in all these conditions are generally <2000 mU/L in an asymptomatic patient [450]. In true prolactinomas, PRL concentrations are usually >5000 mU/L and this results in saturation of antibody–antigen complex formation and falsely low readings from 'the hook effect', with samples needing dilution for accurate quantification in some cases [451]. Macroprolactinomas are more common in boys, and in prepubertal children non-endocrine raised ICP symptoms are more common than signs of hormone excess [452–454]. Galactorrhoea, pubertal delay and amenorrhoea are more common presenting features in older girls. Associated hypothalamo-pituitary endocrine dysfunction is more frequent with larger tumours [453].

In the absence of a neurosurgical emergency, medical monotherapy with dopamine agonists is recommended as first-line treatment for all prolactinomas due to their excellent efficacy, probable preservation of residual hypothalamo-pituitary function and low side effect profile, cabergoline at doses of up to 3.5 mg/week being favoured over bromocriptine [450].

MEN-1 has been associated with larger tumours and more treatment resistance [442, 444], and second-line management includes maximizing cabergoline doses (up to 11 mg/week), surgical resection, radiotherapy and chemotherapy with temozolomide [450, 451, 455]. Knowledge of the maximum safe therapeutic cabergoline

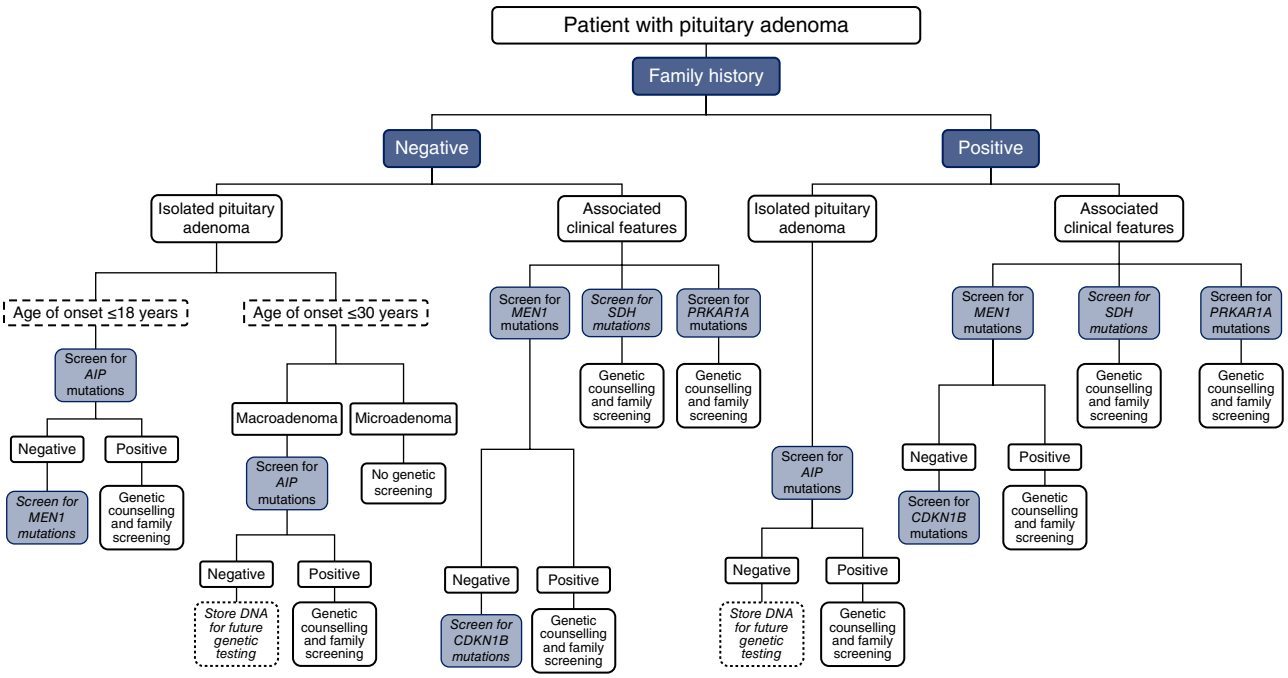

Figure 5.10 Suggested algorithm for genetic screening of pituitary adenomas. *Source:* Taken from Korbonits et al. [446]. Reproduced with permission of John Wiley and Sons.

dose (particularly the long-term risk of cardiac valve abnormalities with long-term cumulative exposure [456]), speed of dose escalation, long-term toxicities of temozolomide and experience in pediatric trans-sphenoidal resections is limited. Long-term tumour- and/or treatment-related morbidities in survivors, including hypopituitarism, obesity, dyslipidaemia, infertility and reduced bone mineral density, have been reported [449, 452, 453].

Corticotrophinomas

Corticotrophinomas account for >70% of pituitary adenomas in the prepubertal age group (<11 years), with Cushing disease caused by an ACTH-secreting pituitary adenoma being the commonest cause of Cushing syndrome in children aged >5 years [440, 457]. Corticotrophinomas are almost always microadenomas [440], but cases of MEN-1-associated macroadenomas have been reported [458]. These tumours usually present with rapid weight gain (94–100%), change in facial appearance (100%), linear growth impairment (96%), fatigue (59–64%), striae (53–64%), hirsutism (38–56%), emotional lability (53%), hypertension (32–50%), acne (44–50%) and headaches (16%) [457, 459, 460]. There is usually a discrepancy in growth with short stature (height < −2 SDS in 40%) being accompanied by an increased BMI [457]. Neurobehavioural changes include poor academic performance and psychotic symptoms [460].

Cushing disease is best diagnosed by confirming the presence of Cushing syndrome (i.e. hypercortisolaemia) with a midnight cortisol concentration of >121 nmol/L (sensitivity 99%, specificity 100%), followed by an overnight high-dose dexamethasone suppression test (sensitivity 97.5%, specificity 100%) [461], although other combinations of the low-dose dexamethasone suppression test and CRH test have been used [457, 460]. MRI may be supplemented with bilateral inferior petrosal sinus sampling (BIPSS), a specialized neuroradiological technique that helps successfully localize the position of the microadenoma [457, 459].

Trans-sphenoidal resection is the first-line treatment for pediatric corticotrophinomas and has superseded bilateral adrenalectomy that carries a significant risk of post-operative Nelson's syndrome [462]. Cure rates are 45–78% but nearly 40% require adjuvant radiotherapy [463–465]. Long-term complications include hypopituitarism, with GH and ACTH deficiency being commoner than other hypothalamo-pituitary endocrine deficits (GH deficiency 44%, ACTH deficiency 38%, TSH deficiency 19%, LH/FSH deficiency 13%, persistent central DI 13% in one case series) [460]. Obesity and insulin insensitivity are recognized long-term morbidities in survivors [449, 457, 459, 460].

Somatotrophinomas

Somatotrophinomas account for 8–15% of all pituitary adenomas in patients <20 years of age [440, 466]. Given their similar origins in pituitary development, a significant proportion co-secrete PRL and TSH. The more commonly recognized form of adult-onset GH hypersecretion leading to acromegaly manifests itself in childhood and adolescence as pituitary gigantism. Growth accelerates, often in association with mild obesity and macrocephaly [438]. Other manifestations of acromegaly are often also present, including acral enlargement (with rapid change in shoe size), frontal bossing and increase in jaw size [467]. Like other pituitary adenomas, a proportion of these tumours are found in association with clinical features of syndromes such as MEN-1, Carney complex and McCune–Albright syndrome [445] but a proportion of these tumours are inherited in an autosomal dominant fashion with incomplete penetrance within families without any other clinical features, a condition known as the FIPA syndrome. 20% of these families have a heterozygous germline mutation in the *AIP* gene, which is associated with larger, more treatment-resistant tumours [446]. High random GH and IGF-1 concentrations, loss of GH pulsatility and failure of GH suppression on an oral glucose tolerance test all point to the diagnosis [438].

Trans-sphenoidal resection remains the treatment of choice with results comparable with that of the adult experience [468–470] but the risk of lifelong panhypopituitarism is high, and a significant proportion of patients requires adjuvant medical therapy usually in the form of somatostatin analogues (e.g. octreotide, lanreotide), dopamine agonists (e.g. cabergoline, bromocriptine) or the GH receptor antagonist pegvisomant, which has shown promising results in acromegalic adults [471]. Radiotherapy has been used with limited effect but carries the risk of second malignancies in oncogenic disorders such as McCune–Albright syndrome and MEN-1, as well as neurocognitive effects [469, 470].

Germinomas

Epidemiology, Histology and Genetics

Germinomas are rare, accounting for only 3–4% of all primary pediatric and young adult CNS tumours, with a particular predilection for adolescents and young adults where the age-adjusted incidence rises from 0.9 cases/million/year to 1.3–2.1 cases/million/year [317, 472]. Boys are affected nearly three times as often as girls, with this distribution being magnified around adolescence (>8:1) [317]. Germinomas are the most frequent CNS tumour seen in chromosome aneuploidies such as Klinefelter and Down syndromes [473]. Intracranial germinomas are a subset of germ cell tumours (accounting for 34% of all such tumours) and resemble their gonadal

counterparts (e.g. ovarian teratoma, testicular seminoma) in secreting α-fetoprotein (AFP) and β-human chorionic gonadotropin (β-hCG), although this is not universal [474].

Clinical Presentation and Investigations

Germinomas grow extremely slowly and clinical and radiological features can often be subtle in onset, with delays in diagnosis of up to 21 years having been reported [475, 476]. Central DI (30–93%) is particularly common at diagnosis, although signs of raised ICP (headache, nausea, vomiting), visual impairment, growth failure and precocious puberty are often seen [475–477].

On MRI, germinomas have a particular predilection for the pituitary stalk and suprasellar regions (32–53%) and the pineal gland (12–57%) such that synchronous pineal and suprasellar lesions (occurring in up to 26% of cases) are pathognomonic [475–478]. Their MRI appearances (T_1- and T_2-isointense with contrast enhancement) cannot easily be differentiated from other causes of a thickened pituitary stalk such as Langerhans cell histiocytosis (LCH) and lymphocytic hypophysitis (Table 5.11), which are frequently associated with central DI and an absence of the posterior pituitary bright spot, making management decisions difficult [479, 480].

The difficulty is compounded by a lack of consensus as to what constitutes an abnormally thickened pituitary stalk in children; one suggestion is to use a threshold thickness of 3.8 mm at the optic chiasm and 2.7 mm at the pituitary insertion, particularly if there are interruptions in the normal smooth tapering of the infundibulum from median eminence to pituitary insertion [299]. Biopsies of the infundibulum to obtain a definitive histological diagnosis can be inconclusive and can lead to panhypopituitarism and central DI [481]. Consequently, various diagnostic algorithms have been proposed for the management of a thickened pituitary stalk, with the presence of central DI, the coexistence of anterior pituitary dysfunction and a progressive increase in infundibular size of >15% from baseline being suggested risk factors requiring more intensive follow-up or biopsy [481, 482].

Treatment and Outcomes

Germinomas have a propensity to metastasize throughout the cerebrospinal fluid but their radiosensitivity has meant that whole neuraxial irradiation has been standard therapy for decades, with OS and PFS rates approaching 100% [473, 477, 478]. Chemotherapy alone has inferior survival [483] but attempts to reduce the irradiation field with adjuvant chemotherapy to preserve cognitive function have shown little reduction in OS [473, 484, 485]. As for other suprasellar tumours, long-term post-treatment hypothalamo-pituitary dysfunction is frequent, with 50–60% of patients experiencing at least one endocrinopathy [477].

Hypothalamic Hamartomas

Epidemiology, Histology and Genetics

Hypothalamic hamartomas are extremely rare congenital (rather than neoplastic) malformations consisting of grey matter heterotopia in the tuber cinereum and inferior hypothalamus [297, 298, 486]. Given their neurodevelopmental origin, their true prevalence is unknown but is estimated to occur in every 1 in 50,000–1 million individuals [487–489]. The hamartomatous mass itself may only be a sign of more widespread cerebral cortical dysplasia putting patients at risk of seizures. Rarely, hypothalamic hamartomas are associated with the Pallister–Hall syndrome, an autosomal dominant disorder caused by mutations in the *GLI3* gene resulting in polydactyly and other midline defects (imperforate anus, bifid epiglottis, panhypopituitarism and dysmorphic facies) [488, 490]. Hypothalamic hamartomas may also be associated with *SOX2* mutations in humans [43].

Clinical Presentation and Investigations

Seizures are usually the first presentation and tend to occur in infancy to early childhood between 6 weeks and 5 years [489, 491–493]. The seizures are classically gelastic ('laughing' seizures) and may be difficult to recognize initially but evolve into multiple, more severe seizure types, alongside the other two classical components of the clinical triad – central precocious puberty and developmental delay [486]. The evolving seizures are often unresponsive to anti-epileptic drug therapy and accompanied by worsening behavioural and psychiatric co-morbidities.

Silent endocrine dysfunction (particularly GH and TSH deficiencies) is common at diagnosis and may be missed but some patients present with central precocious puberty and never develop seizures [494]. On MRI, hamartomas appear as isointense lesions that do not exhibit contrast enhancement [297, 298].

Treatment and Outcomes

The intractability of the seizures has led to the use of various surgical or stereotactic radiosurgical techniques to remove or ablate epileptogenic foci, with variably reported success on remission of seizures and behavioural disturbances but more modest improvements in cognitive function [486, 487, 495–497]. GnRH agonist therapy is the obvious choice of medical treatment for the precocious puberty but some case reports indicate success with surgical resection of the hamartoma with little late-onset endocrinopathy [498]. Other authors are more cautious however, and post-treatment hypothalamo-pituitary dysfunction, including central DI, SIADH and hypothalamic obesity, has been observed frequently in larger cohort studies [494, 499, 500].

Miscellaneous Cystic Lesions

Rathke's Cleft Cysts

Rathke's cleft cysts are benign cystic remnants of the Rathke's pouch that fail to involute during pituitary development; they are lined by a single layer of columnar or cuboid epithelium arising from the intermediate lobe but often extending superiorly [297]. They are often found incidentally without any associated symptoms (being present in 11% of autopsy cases [501]) but can become symptomatic, with hypopituitarism and hyperprolactinaemia being commoner presenting features than visual disturbances or headache [502, 503]. Differentiating Rathke's cleft cysts from craniopharyngiomas is not always easy, particularly in the absence of calcification because the cystic fluid exhibits variable signal intensities depending on its contents. Surgical marsupialization is the treatment of choice with low recurrence rates; more aggressive surgical resection is usually associated with higher rates of hypopituitarism [504, 505].

Arachnoid Cysts

Arachnoid cysts are collections of cerebrospinal-like fluid surrounded by a wall of arachnoid structures thought to arise by splitting or duplication of the arachnoid membrane during development. 16% of these cysts are found in the suprasellar region with only rare cases being intrasellar [506–508]. Common presenting features are usually related to raised ICP, particularly macrocephaly with hydrocephalus, but visual impairment, hypopituitarism and developmental delay occur [508, 509]. Central precocious puberty is seen in up to a third of patients [510]. Endoscopic fenestration is the treatment of choice with good resolution of macrocephaly, developmental delay and preoperative seizures [509, 511, 512].

Infiltrative and Inflammatory Disorders

Langerhans Cell Histiocytosis

Epidemiology and Histology

LCH, previously known as 'histiocytosis X', is one of three major histiocyte disorders and involves clonal proliferation of bone marrow-derived antigen-presenting ('Langerhans') cells that accumulate in various organs causing either single- or multi-organ dysfunction [513]. It is rare with an incidence of 2.6–8.9 cases/million/year mostly in infants <1 year (9.0–15.3 cases/million/year). The median age at diagnosis is 2–3.8 years, with no sex predilection [514–517]. The clinical features depend on the organs involved and range from a single self-healing cutaneous lesion to fatal multi-organ disease, particularly if the liver, spleen, lungs and haemopoietic system ('risk' organs) are involved [513]. Multisystem involvement occurs in 27–56% of cases [514–516, 518, 519].

Clinical Features

In 25% of cases, LCH involves the hypothalamo-pituitary region, particularly the infundibulum, and almost invariably leads to central DI (previously known as Hand–Schüller–Christian disease if associated with orbital and bony lesions) [514, 515, 517, 520, 521]. Central DI is particularly frequent in the presence of multi-organ LCH, particularly if involving 'risk' organs, craniofacial bones, the gastrointestinal tract, skin or genitalia [521, 522], and can often precede the diagnosis of LCH itself [523]. Infiltration of the anterior pituitary is less common but can also lead to growth retardation and/or panhypopituitarism [513]. Over time, deficiencies in GH (9–15%), TSH (3–4%), LH/FSH (3–5%) and ACTH (<2%) may evolve [520, 524, 525]. Anterior pituitary deficits can occur in the absence of DI [520, 525].

Investigations

LCH is one of the differential diagnoses of a thickened pituitary stalk and radiological appearances cannot be differentiated easily from that of germinomas or hypophysitis (Table 5.11). The thickened stalk can progress to frank space-occupying tumours, and the presence of DI is associated with an absent posterior pituitary bright spot [526]. LCH is the commonest underlying diagnosis in patients with central DI and an intracranial mass – 70% of patients in one such series had this diagnosis [527].

MRI appearances do not correlate with clinical recovery and DI persists in all cases, regardless of treatment. 75% of LCH cases with DI show a thickened infundibulum at diagnosis but only 24% persist after 5 years [521, 528]. Radiological appearances do not consistently predict the evolution of anterior pituitary dysfunction, which does not reverse with treatment [525, 528–530].

Treatment and Outcome

Treatment is dependent on the organs involved. LCH localized to skin and bone may be treated by biopsy and/or curettage, bearing in mind that such lesions also have the propensity for self-healing [531]. Other options include intralesional steroids [532], focal radiotherapy [522] and indomethacin [533] for bone lesions and topical nitrogen mustard for skin lesions [534]. A comparison of systemic chemo- and/or radiotherapy with local therapy for multiple 'CNS-risk' bone lesions (i.e. 'single organ' but involving craniofacial bones and a high risk of DI) showed no difference in 5-year event-free survival, suggesting that local therapies, which have less side effects, may be appropriate [535].

Several prospective randomized clinical trials to determine the best chemotherapeutic protocol for treatment of multi-organ LCH, which in itself is a risk factor for central DI and long-term hypothalamo-pituitary

dysfunction, have been reported. The outcome of the LCH-I to LCH-III studies has shown that standard care with 12 months of vinblastine and oral prednisolone for patients with and without risk organ involvement achieves the lowest 5-year reactivation rates of 27 and 37%, respectively, with OS being 84% for those with risk organ involvement and 99% for those without [536].

Other agents that have been used include cytarabine, cladribine and clofarabine, particularly for refractory cases [537–539]. Allogeneic haematopoietic stem cell transplantation has been attempted with 3-year survival rates of up to 77% [540]. Mutations in the *BRAF*, *ARAF* and *MAP2K1* oncogenes have been identified and opened up possibilities for more targeted treatments such as B-Raf inhibitors [541]. It is worth noting that central DI and other endocrinopathies are not reversed by any of the treatment regimens, impact significantly on long-term quality of life in survivors [522, 542] and predict a poorer prognosis [520]. GH replacement does not affect LCH reactivation or survival [529].

Lymphocytic Hypophysitis

Epidemiology and Histology

Hypophysitis refers to the inflammation of the pituitary gland and can be primary or secondary, the latter arising from infection, systemic disease or irritation from adjacent lesions (Table 5.13). Primary hypophysitis is classified by histology into three major subtypes – lymphocytic, granulomatous and xanthomatous (with some authors additionally recognizing the necrotizing and xanthogranulomatous subtypes) [543].

The lymphocytic subtype is commonest and is further subdivided by anatomy into lymphocytic adenohypophysitis (affecting the anterior pituitary only), infundibuloneurohypophysitis (affecting the posterior pituitary) or panhypophysitis [544]. The aetiopathology of primary lymphocytic hypophysitis is thought to be immunological, given its well-recognized association with autoimmune diseases (e.g. Hashimoto's thyroiditis, Grave's disease, Crohn's disease, Sjögren's syndrome, autoimmune polyendocrinopathy syndromes), pregnancy and the presence of a variety of autoimmune antibodies, including those against the pituitary gland itself [544–548]. Regardless of histology, the underlying immunological mechanism seems to involve CD8+ T-cell-mediated cytotoxicity [545].

Lymphocytic hypophysitis is exceedingly rare in children. A recent review identified only 96 cases occurring in children <18 years [298]. The epidemiology in children differs slightly from adults – there is less of a female sex predilection (in adults the male/female ratio is 1:8) and the association with autoimmune disorders is lower (6 vs. 16% in adults) [546, 549].

Table 5.13 Causes of hypophysitis.

Primary hypophysitis
Lymphocytic
Granulomatous
Xanthomatous
(Necrotizing)
(Xanthogranulomatous)
Secondary hypophysitis
Local lesions
Germinomas
Craniopharyngiomas
Pituitary adenomas
Rathke's cleft cysts
Systemic disease
Langerhans cell histiocytosis
Sarcoidosis
Wegener's granulomatosis
Tuberculosis

Clinical Features and Investigations

Headaches are the commonest presenting feature in adults [546] but children are more likely to present with symptoms and signs of hypothalamo-pituitary dysfunction, 85% with central DI [549]. In adults, the commonest anterior endocrine deficiency is that of ACTH, followed by TSH, LH/FSH, PRL and GH [544, 546]. Autoimmunity against the intermediate lobe, or to corticotroph-specific transcription factors such as *TPIT*, is thought to be the reason for the frequency of ACTH deficiency in this condition [548, 550]. In children, the frequency of endocrine dysfunction is more typical of that found in most suprasellar lesions, GH (76%), LH/FSH (32%), TSH (29%) and ACTH (20%), with 11% having panhypopituitarism [549].

Lymphocytic hypophysitis, like LCH, is a differential diagnosis for the thickened pituitary stalk (Table 5.11). In a case series of 11 adult and pediatric patients biopsied for such a neuroradiological appearance, 27% had evidence for hypophysitis [551]. The appearances of hypophysitis may also herald the development of an indolently growing germinoma that may not become clinically apparent for several years [552, 553].

Treatment and Outcomes

The natural history of lymphocytic hypophysitis is spontaneous resolution and treatment is therefore symptomatic. In adults, raised ICP or mass-related symptoms and signs are usually relieved surgically by the trans-sphenoidal approach [544]. Alternatively, glucocorticoids in the form of methylprednisolone or prednisolone (both of which concurrently replace any secondary cortisol deficiency if present) and steroid-sparing agents such as azathioprine and methotrexate have been used with variable results [544]. Given the difficulties in

performing trans-sphenoidal surgery in young children and the risks of causing further endocrinopathies, the latter option is preferred but, regardless of resolution, the majority of hypothalamo-pituitary dysfunction does not resolve [549].

Sarcoidosis

Sarcoidosis affects the CNS in 5–15% of patients [554]. The hypothalamus and pituitary are rarely affected, with central DI being the most frequently reported endocrinopathy in up to 78% of patients [555]. This is followed by deficits in LH/FSH, TSH, GH and ACTH, with hyperprolactinaemia and panhypopituitarism being relatively common [555, 556]. The disease is rare in children [556–558]. Raised angiotensin-converting enzyme (ACE) concentration is suggestive of the diagnosis [558]. The prognosis of neurosarcoidosis is poor, despite treatment with glucocorticoids or other immunosuppressive agents. Hypopituitarism generally persists or evolves, although some cases do recover [555, 556].

Traumatic Brain Injury

Epidemiology and Histology

The incidence of TBI in children is ~180 per 100,000 per year, with around 5.6 per 100,000 being in the most severe category requiring intensive care and almost one-third requiring neurosurgery [559]. The incidence increases with age with a male predominance, peaking in adolescence and early adulthood, with rates of >250 per 100,000 [560]. The causes of head injury in children are more diverse than in adults, with falls, pedestrian and road traffic accidents and sport-related injuries occurring from early childhood into adolescence, while non-accidental injuries, including shaken baby syndrome, are more frequent in infancy and early childhood [559, 561].

Despite the fact that TBI has long been known to be associated with hypopituitarism, with numerous studies reporting a prevalence of between 28 and 69% in adult TBI survivors [562], pediatric epidemiological studies have been less detailed. Although clinical outcomes in pediatric survivors of TBI have often been thought to be better, initial case reports and case series indicate higher rates of hypopituitarism, with 80–85% experiencing deficiencies in GH and LH or FSH [562, 563]. Prospective studies indicate that this prevalence is lower, with between 16 and 42% of pediatric survivors experiencing some degree of hypopituitarism [564–566].

Despite being protected within the sella turcica, the rich neurovascular network between the hypothalamus and pituitary, particularly the infundibulum, is vulnerable. The superior hypophyseal arteries and portal capillaries surrounding the pituitary gland and infundibulum are susceptible to traumatic rupture and haemorrhage leading to ischaemia and infarction. Pituitary infarction can also arise from tissue swelling and oedema, resulting in compression of the blood supply to the gland within the narrow entry point into the sella turcica. Post-mortem studies have demonstrated lesions consistent with haemorrhage, infarction, necrosis and fibrosis of the hypothalamus and anterior pituitary gland, with occasional transection of the infundibulum itself [567, 568]. The particular susceptibility of the GH axis may be related to the fact that somatotropic cells are located in the lateral portions of the pituitary gland, away from the capsule, and are therefore dependent on the portal vasculature, making them most vulnerable to disruption. The peripheral pituitary cells, such as those secreting ACTH and TSH, are located in the medial portion of the pituitary gland and have a dual source of blood supply from both the portal vessels and the capsular anterior pituitary artery [569].

Clinical Presentation and Investigations

Hypothalamo-pituitary dysfunction may be identified in the first few days to weeks after injury (acute phase) or may develop over time. The notable overlap between chronic neuropsychological symptoms experienced in general by all TBI survivors and those of hypopituitarism may mean that some of these clinical features are overlooked and the diagnosis of endocrine deficits missed, with delays in diagnosis by up to 42 years [570]. Various attempts have also been made to correlate the severity of the original injury with the incidence of endocrine dysfunction using the Glasgow Coma Scale or the Glasgow Outcome Score [571], but no clear relationship has been found [564, 566, 572].

Alterations in endocrine function in the acute phase may reflect the adaptive response to acute illness. As with post-neurosurgical patients, clinically significant hypothalamo-pituitary dysfunction mainly involves regulation of fluid and electrolyte balance (central DI, SIADH, cerebral salt wasting) and ACTH deficiency. Many of these hormone deficiencies are transient and do not necessarily predict the development of permanent hypopituitarism [573]. In adult studies, gonadotropin deficiency appears the commonest in the acute phase, 40–80% of patients being affected [572, 573]. Assessment of the gonadotropin axis is more difficult in children and the frequency of true gonadotropin deficiency in this period is unknown.

In a prospective subcohort analysis, low T_3 concentrations were found in 23% of patients in the acute phase, with 10% having cerebral salt wasting and one patient having central DI [353]. A significant proportion of these patients (36%) had ACTH deficiency, although the clinical significance of this and the need for supplementation

are as yet unknown [354]. Critical illness activates the hypothalamic–pituitary–adrenal axis with concurrent reduction in the concentration of cortisol-binding globulin, an increase in free cortisol concentrations and tissue sensitivity to glucocorticoids [574]. Concurrent existence of central DI may complicate acute fluid management and be a marker of poor prognosis and mortality: of 19 children with central DI (12 with TBI), only three survived the acute insult [575].

Hypothalamo-pituitary endocrine dysfunction may persist or recover from the acute phase and new deficits may evolve. GH deficiency was the commonest (85%), followed by deficiencies in LH/FSH (80%), TSH (75%), ACTH (55%) and ADH (10%) [562, 563]. More recent prospective data have indicated that the incidence of such endocrine dysfunction may not be as high as previously thought, although the frequency is still significant, 30–61% of children having long-term dysfunction 1–2 years after the initial insult, consisting of deficiencies in GH (4–42%), ACTH (8–34%), LH/FSH (17%) and TSH (6–12%) [564–566, 576]. The posterior pituitary seems more robust in the long term (incidence of central DI 4.3%) with recovery of the majority of acute changes [576]. Occasional cases of central precocious puberty have been reported [566, 576].

Management

Consensus guidelines have been produced suggesting that all TBI patients should be evaluated prospectively at the time of initial insult and three and twelve months thereafter [577]. Assessment should include a morning cortisol measurement and paired osmolalities in the presence of a history of polyuria. Results from baseline testing guide the necessity for further dynamic testing and supplementation as needed. In children and adolescents, the period of transition is important with a multidisciplinary approach being advocated [563]. The long-term outcome of TBI appears to be more favourable in children compared with adults, although the negative impact of endocrine dysfunction on neurocognition and quality of life is only beginning to be understood [578, 579].

CNS Infection

Epidemiology and Aetiology

Hypothalamo-pituitary dysfunction is rare but has been reported after meningitis and encephalitis. Tuberculous meningitis has long been known to result in hypopituitarism in both adults and children with 20–50% of patients being affected [580, 581]. A wide variety of other organisms have been associated with hypothalamo-pituitary endocrine deficits, although at a slightly lower frequency (Table 5.14) [582, 583]. The true frequency of this complication is probably lower, given the selection bias of many of the studies. Tuberculous meningitis can be complicated by the development of hypothalamo-pituitary tuberculomas [584], but brain abscesses of bacterial origin can also result in endocrinopathies [583].

Table 5.14 Known reported causative agents of CNS infection-related hypopituitarism.

Mycobacterium tuberculosis
Bacterial
Group B streptococcus
Haemophilus influenzae
Streptococcus pneumoniae
Borrelia burgdorferi
Protozoan/parasitic
Trypanosoma cruzi
Cysticercosis
Fungal
Cryptococcus sp.
Aspergillus sp.
Viral
Cytomegalovirus
Coxsackie virus
Herpes simplex virus
Enterovirus
Influenza A
Varicella zoster
Tick-borne encephalitis

Clinical Features and Investigations

Given the frequency of endocrine deficits in tuberculous meningitis, more detailed data on the relative frequency of dysfunction in the various hypothalamo-pituitary axes are available. In a prospective study of 49 patients, 20% of patients were found to have endocrinopathies with GH deficiency being commonest (70%), followed by deficiencies in LH/FSH (50%), ACTH (10%) and hyperprolactinaemia (10%) [360].

MRI findings ranged from contrast-enhancing suprasellar lesions involving hypothalamus, pituitary and infundibulum to pituitary atrophy and ventricular dilatation. A study of 75 patients indicated similar relative frequencies (LH/FSH [20–36%], ACTH [31%], TSH [25%]) but had a higher incidence of hyperprolactinaemia (49%) and did not look for GH deficiency. MRI findings were similar, with two patients having tuberculomas [360].

The diagnosis of hypothalamo-pituitary tuberculomas is difficult because less than a third have a history of previous or concurrent TB infection [585, 586]. MRI appearances are not easily differentiated from other causes of granulomatous hypophysitis such as sarcoidosis or Wegener's granulomatosis with lesions often being negative for acid-fast bacilli [586, 587]. Frequent symptoms include headache and visual impairment, with over 60%

of patients experiencing anterior pituitary dysfunction and 10% having central DI [584].

In non-mycobacterial CNS infections, the frequency of endocrine dysfunction appears to be lower. In a prospective study by Schaefer et al. [582], only 21% of patients appeared to have ACTH deficiency, and 11% of patients had hypogonadotropic hypogonadism, with no abnormalities reported in the GH, TSH, PRL or ADH axes. Dhanwal et al. [583] demonstrated similar deficiencies in ACTH (23%), LH/FSH (23%) and TSH (3%) with 30% developing hyperprolactinaemia and none developing central DI.

Treatment and Outcomes

Treatment depends on the causative organism. BCG vaccination programmes and better anti-tuberculous drug therapy have resulted in a reduction in incidence in tuberculous meningitis and hypothalamo-pituitary dysfunction in the developed world. Treatment of tuberculomas is poorly defined but surgical drainage has been advocated in combination with anti-tuberculous drug therapy and steroids [584]. The endocrine deficits observed in all CNS infections may be transient, recurrent or permanent and therefore require careful follow-up [584, 588–590].

Haemochromatosis

Epidemiology and Aetiology

Haemochromatosis can be either primary due to mutations in genes directly regulating iron absorption and storage or secondary (due to other genetic, acquired and iatrogenic conditions resulting in iron overload [Table 5.15]).

Primary haemochromatosis is caused by mutations in a variety of genes that cause a deficiency in hepcidin or lead to disruption in its function. Hepcidin is a liver-derived hormone that is increased in response to iron overload and inflammation and is reduced in iron deficiency, hypoxia and ineffective erythropoiesis; it signals to the gastrointestinal tract to reduce its release of absorbed iron into the plasma via the iron transport protein ferroportin 1 [591, 592]. Changes in genes causing a disruption in this signalling pathway (*HFE*, *HJV*, *HAMP*, *TfR2*, *SLC40A1* [ferroportin 1]) result in a pathological increase in non-transferrin-bound iron, which is then taken up by the liver, pancreas, pituitary gland, gonads, heart, joints and skin [591, 592]. While *HFE*-related haemochromatosis is the commonest inherited primary iron overload disorder in adults, it does not present in childhood, where the much rarer non-*HFE*-related mutations are more prevalent.

Secondary haemochromatosis is probably more common in childhood and results from congenital and acquired disorders that indirectly affect the iron regulatory pathway. The commonest of these are the iron-loading anaemias, with β-thalassaemia major and intermedia being the most frequent. The prevalence of all forms of β-thalassaemia is 1 in 100,000 worldwide but with significantly higher frequencies in the Mediterranean, Middle East, Central Asia, India and the Far East [593]. Iron overload resulting from repeated blood transfusions is a major cause of secondary haemochromatosis in these conditions but hypoxia, increased soluble *HJV* and erythropoietin expression all result in decreased hepcidin expression [591]. Other potential groups at risk of secondary iron overload include children who receive multiple transfusions for other reasons, including chemotherapy-induced immunosuppression, although the effect on endocrine function remains undefined [594]. Secondary hypothyroidism has been described in neonatal haemochromatosis [595].

Table 5.15 Primary and secondary causes of haemochromatosis.

Primary haemochromatosis
HFE related (type 1)
Non-HFE related
Juvenile haemochromatosis (type 2: haemojuvelin/hepcidin mutations)[a]
Transferrin receptor 2 haemochromatosis (Type 3)[a]
Ferroportin haemochromatosis (type 4)[a]
Secondary haemochromatosis
Iron-loading anaemias
Thalassaemias (β-thalassaemia major and intermedia)[a]
Sideroblastic anaemias[a]
Chronic haemolytic anaemia[a]
Aplastic anaemia[a]
Pyruvate kinase deficiency[a]
Chronic liver disease
Hepatitis C infection
Non-alcoholic fatty liver disease
Alcoholic liver disease
Porphyria cutanea tarda
Iatrogenic
Repeated red blood cell transfusions[a]
Long-term haemodialysis[a]
Miscellaneous
Acaeruloplasminaemia
African iron overload
Neonatal haemochromatosis[a]

[a] Indicates disorder seen in children.

Clinical Features and Investigations

Excess non-transferrin-bound iron is avidly taken up by multiple endocrine organs, including the pancreas (resulting in diabetes), gonads (resulting in primary hypogonadism) and thyroid gland (resulting in primary

hypo- or hyperthyroidism) [591, 596, 597]. The hypothalamus and pituitary gland are also susceptible to iron deposition, with the gonadotropin axis (both GnRH and LH/FSH) being particularly vulnerable [598, 599]. In a cohort of 17 patients with secondary haemochromatosis, 35% had delayed puberty, with all 14 patients who were in puberty having abnormal LH pulsatility compared with healthy controls [600]. 35% of patients also had GH deficiency. 40–59% of thalassaemic patients have hypogonadism manifesting as pubertal delay or arrest, primary and secondary amenorrhoea or infertility and 33–36% have growth failure [601–603], with the growth deficit becoming apparent particularly in adolescence [604].

The predilection for the hypothalamic–pituitary–gonadal axis is partly explained by an immunocytochemical study of pituitary adenomas by Atkin et al. [605], which demonstrated that only gonadotrophinomas show immunopositivity for the transferrin receptor. Similar studies in rats demonstrate that transferrin receptors are expressed only in somatotrophs and gonadotrophs [606]. In non-endocrine organs, iron accumulation can result in liver cirrhosis, cardiomyopathy, arthritis and skin hyperpigmentation [591, 592].

MRI appearances of pituitary iron overload demonstrate a reduction in T_2-weighted signal intensity. The best technique to visualize this appearance is the gradient-echo T_2^*-weighted technique, with a reduction in pituitary-to-fat signal intensity ratio <1.1 inversely correlating with ferritin concentrations [607, 608]. The height of the pituitary gland may also be reduced, possibly due to destruction of gonadotrophs from iron deposition [609]. An elevated transferrin saturation is a more sensitive and specific screening test than a raised ferritin, which is a better indicator of disease severity, especially if >1000 µg/L [591, 592].

Treatment and Outcomes

In primary haemochromatosis, venesection is the primary treatment, aiming to keep serum ferritin <50–100 µg/L [591, 592]. This treatment is not usually appropriate in secondary haemochromatosis because of the common association with anaemias and iron chelation with subcutaneous desferrioxamine, and oral deferasirox is used to increase urinary and faecal excretion, resulting in improved long-term outcomes and reduced mortality [610–612]. There is some evidence that high-dose iron chelation therapy can reverse hypothalamo-pituitary and other end-organ endocrinopathies [613] but over-chelation can also result in growth retardation due to the toxic effects of desferrioxamine on the spine [614]. A recent randomized trial has suggested that deferasirox demonstrates a better safety profile in terms of growth and puberty [615]. Careful dosage of iron chelation is required to avoid both under- and overdosing, with some authors suggesting that chelation should not start till after 2–3 years of age, with starting doses of desferrioxamine of 40 mg/kg/day, increasing to a maximum of 50 mg/kg/day 5–7 days a week or deferasirox at 20 mg/kg/day increasing to 40 mg/kg/day with careful monitoring of growth [616, 617].

Psychosocial Deprivation

Psychosocial short stature – the association between extreme psychosocial distress in children, often from severe emotional abuse or neglect, and short stature – was first described by Talbot et al. [618] in a cohort of 51 children, where heights were similar to untreated children with hypopituitarism. A subsequent case series of 13 children described several clinical features unique to this syndrome – the absence of signs of malnutrition, abnormal behaviour (including hyperphagia, polydipsia, night roaming, encopresis), loose stools and developmental delay [619]. Three-quarters of these children demonstrated functional GH deficiency, with all of them recovering on repeat testing when removed from their home environment [620].

There are two forms of this syndrome lying along a spectrum. 'Type 1' psychosocial short stature is more common, is present in younger children (<2 years) and is associated with physical neglect and clear signs of malnutrition and anorexia. 'Type 2' occurs in older children (>3 years) in association with emotional abuse and/or neglect with the more classical features as described above [621]. Other clinical features that have been described include abdominal distension, gorging with subsequent vomiting, enuresis, sleep disturbances (with a reduction in deep slow wave sleep), pain agnosia, elective mutism and intermittent swelling of the hands and feet [620–624].

Apart from transient GH deficiency, patients may have delayed puberty and delayed bone age, with X-rays often showing growth arrest lines [625]. The GH deficiency typically reverses within two to three weeks of removal from the home environment [626]. Growth does not respond to GH treatment [627]. IGF-1 concentrations are low but respond to GH stimulation [628]. Thyroid function tests are normal but ACTH reserve is variable and suggests a hypothalamic CRH deficit [620]. Skuse et al. [624] have proposed diagnostic clinical criteria for this disorder and found that the presence of hyperphagia is a strong predictive factor for GH reversibility and the likelihood of a psychosocial component in a short child. Despite positive changes to the home environment and recovery of endocrine function, the prognosis for emotional stability and final height is not good [621, 629].

Investigation of Hypopituitarism

The diagnosis of hypopituitarism is based upon a combination of clinical assessment, looking for the presence of not just endocrine-related symptoms and signs but also for other features related to the presence of a congenital syndrome or a suprasellar mass (Tables 5.5 and 5.12). Auxology (height, weight, BMI and their corresponding SD scores) and pubertal assessment are mandatory. If available, prior growth curves from community screening may indicate the presence of growth failure or rapid weight gain in the months preceding diagnosis. For instance, in craniopharyngiomas, early changes in weight and BMI have been shown to precede the diagnosis of a suprasellar mass by several months and may be predictive of future hypothalamic obesity [318, 630]. The presence of precocious, delayed or arrested puberty supported by an inappropriately delayed or advanced bone age may also help predict future growth potential and determine if intervention is needed to achieve this.

Confirmation of hypopituitarism is a stepwise process, usually beginning with a baseline pituitary function screen, followed by dynamic pituitary function tests (Table 5.16). Normal hormone secretion is dependent upon the presence of an intact hypothalamic–pituitary–target gland axis. Dynamic tests aim to stimulate the relevant axis if hormone deficiency is suspected and to suppress it to test for hormonal excess. Discussion of the different forms of dynamic endocrine tests is beyond the scope of this chapter, but several key points should be noted:

- Basal pituitary hormone screening helps risk-stratify patients who require prioritizing for dynamic testing before definitive treatment of a suprasellar tumour, particularly to establish the status of the hypothalamic–pituitary–adrenal and ADH axes in order to avoid the fatal consequences of uncorrected cortisol insufficiency and/or central DI during surgery. In this situation, early morning cortisol and ACTH concentrations should be measured, particularly prior to administration of high-dose dexamethasone for peritumoral oedema.
- In some cases, dynamic function testing is not required – for instance, a low concentration of free T_4

Table 5.16 Investigation and management of hypo- and hyperpituitarism: for suprasellar tumours, these measurements must be taken before administration of dexamethasone for peritumoral oedema.

Pituitary hormone axis	First-line investigation	Second/third-line investigation for deficiency	Second/third-line investigation for excess	Treatment of deficiency
GH	IGF-1, IGF-BP3	GH provocation tests (insulin (gold standard), glucagon, clonidine, arginine-GHRH[a]) Overnight GH profiling	Oral glucose tolerance test Overnight GH profiling	GH 0.5–1.0 mg/m^2/day
LH/FSH[b]	LH, FSH, oestradiol/ testosterone	GnRH stimulation test	Not needed in children – diagnosed on clinical and baseline biochemical features alone	Sex-appropriate oestradiol/ testosterone supplementation in escalating doses
TSH	TSH, free T_4 ± free T_3	Not needed	Not needed	Levothyroxine titrated against thyroid function tests (starting dose 3–15 mcg/kg/day)
ACTH	Morning (7–9 am) cortisol, ACTH[c]	Insulin tolerance test (gold standard) Standard/low-dose Synacthen tests 24 hour cortisol profile	2400 and 0800 cortisol and ACTH 24 hour urinary free cortisol Overnight/low-dose/high-dose dexamethasone suppression tests BIPSS	Hydrocortisone 8–15 mg/m^2/ day
PRL	PRL	Not needed	Macroprolactin Serial dilutions for hook effect	Not needed
ADH	Early morning paired urine and plasma osmolalities	Water deprivation test DDAVP test	Not needed	DDAVP titrated against osmolalities and clinical symptoms

[a] The arginine-GHRH test cannot be used to diagnose GHRH deficiency.
[b] In infants <6 months or boys >9 years and girls >8 years of age.
[c] For suprasellar tumours, these measurements must be taken before administration of dexamethasone for peritumoral oedema.

in the presence of an inappropriately low or normal concentration of TSH is sufficient for the diagnosis of secondary or tertiary hypothyroidism, and an additional TRH stimulation test neither differentiates the two nor changes clinical management [631].

- The timing of basal and dynamic function tests may be important – testing for hypogonadotropic hypogonadism is not useful outside of the mini-puberty (up to age of 6 months) and pubertal phases (boys aged >9 years, girls aged >8 years) as the hypothalamic–pituitary–gonadal axis is quiescent outside these periods [632].
- In some cases, 24 hour (cortisol) or overnight (GH) hormone profiling to determine spontaneous hormone secretion may be necessary to detect more subtle endocrine deficits that would otherwise appear to be normal based on values obtained from artificial stimulation tests [633].
- The interaction between the different hypothalamo-pituitary axes is relevant in interpreting endocrine tests – for instance, cortisol sufficiency is required to permit renal free water clearance via inhibition of ADH secretion, so ACTH deficiency may mask coexistent central DI until glucocorticoid replacement is initiated.
- If a suprasellar tumour is suspected or has already been confirmed by neuroimaging, additional investigations should also be performed to determine if the mass is responsible for secreting AFP and/or β-hCG (germinomas), PRL (prolactinomas), GH (somatotrophinomas) or ACTH (corticotrophinomas). Occasionally, measurement of cerebrospinal fluid AFP and β-hCG concentrations may be needed to support the diagnosis of a germinoma. Evidence of tumour secretion of various molecules can help avoid the need for a diagnostic biopsy or unnecessary surgical resection, since such procedures carry significant risks of further damaging the hypothalamo-pituitary axis.

All patients with documented hypopituitarism warrant an MRI. The role of genetics in congenital hypopituitarism remains to be established and is offered only on a research basis. Appropriate mutational screening is an important adjunct to management because understanding the pathophysiological process may predict prognosis, improve early diagnosis and management and assist with counselling the family. Some mutations may assist in the differential diagnosis – *PROP1* mutations, for instance, are associated with suprasellar masses and may help to reassure families of the absence of a brain tumour and circumvent the need for surgery. Given the variable penetrance of many dominant mutations, prenatal diagnosis should not be offered until understanding the genetic basis of many of these conditions has advanced significantly. Whole exome and whole genome sequencing technologies are currently offered on a research basis and may further improve understanding of the genetic aetiology of these conditions.

Management of Hypopituitarism

The mainstay of treatment is replacement of the appropriate hormones. In both congenital and acquired hypopituitarism, it is crucial to recognize the potential for evolution of endocrine deficits over time and all patients with documented endocrine dysfunction require lifelong follow-up with appropriate transition into adulthood.

Growth and puberty should be monitored and GH deficiency treated with rhGH until linear growth ceases, although there is increasing evidence for the use of continued GH treatment into adulthood due to its possible metabolic effects on body composition and bone mineral density. In tumour survivors, GH therapy in replacement doses is not associated with an increased risk of recurrence or progression [367, 383, 396, 416], although some evidence suggests that there may be a small increased risk of second primary neoplasms but this is not completely clear [634]. We therefore advocate minimum effective doses for GH replacement, aiming for normal IGF-1 concentrations and an age-appropriate height velocity.

Treatment of coexistent precocious puberty with GnRH analogues can help restore adult height if commenced early but the decision artificially to delay puberty should be decided on an individual basis. Conversely, in the face of coexistent hypogonadotropic hypogonadism, it is not unusual to commence GH supplementation at least six months before sex steroid replacement, the timing of which again should be tailored to the individual child. We do not advocate delaying commencement of the latter beyond the usual pubertal age (~12 years in girls and 14–15 years in boys), given the long-term benefits on bone mineral accretion [635–637]. Precocious puberty does not preclude the evolution of subsequent hypogonadotropic hypogonadism, and children should continue to be monitored carefully after cessation of GnRH analogue therapy to ensure normal pubertal progress [416].

TSH deficiency is easily replaced with levothyroxine supplementation, with dose titrations being based entirely on free T_4 concentrations and not TSH. Some clinicians favour keeping free T_4 concentrations in the upper half of the normal range, particularly given the risk of hypothalamic obesity in many forms of hypopituitarism but before commencing replacement, pre-existing ACTH deficiency should be detected to avoid the risk of precipitating a hypocortisolaemic crisis.

Replacement doses of hydrocortisone should be given at least three times daily and titrated in the growing child against trough concentrations using a 24 hour cortisol day curve. Doses should be doubled or even trebled in the face of illness, with patient education on how to administer emergency intramuscular hydrocortisone (doses <1 year 25 mg, 1–5 years 25–50 mg, >5 years 100 mg) and correct hypoglycaemia during crises.

Management of central DI, particularly in patients with coexisting ACTH deficiency and/or hypothalamic adipsia (e.g. in some post-surgical suprasellar tumour patients), is complex and needs specialist care. Untreated patients with an intact thirst axis and free access to fluid are able to maintain euvolaemia and eunatraemia by adjusting their oral intake appropriately. DDAVP treatment aims to provide the patient with a relatively normal quality of life by reducing the burden of polyuria and polydipsia. Doses should be titrated against trough paired plasma and urine osmolalities, and treatment should ideally be started as an inpatient. Treatment should generally err on the side of underdosing, since overdosing DDAVP can result in hyponatraemia and rapid fluid shifts that are difficult to correct safely due to the risk of cerebral oedema or even death. Patients with hypothalamic adipsia require a strict fluid intake regimen to maintain euvolaemia.

Conclusion

The hypothalamo-pituitary neuroendocrine network can be disrupted by congenital causes or acquired later in life due to anatomical damage from suprasellar masses or their treatments, TBI, infiltration, infection or inflammation leading to various syndromes of endocrine deficits or hormonal excess. Many genetic causes have been discovered, with several single-gene mutations identified. Despite this, the vast majority of congenital hypopituitarism remains idiopathic and probably involves multiple genes and environmental factors. Regardless of cause, hypopituitarism can potentially evolve over time with deficits recurring and/or resolving, mandating lifelong follow-up of such patients.

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6

Disorders of Growth

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KEY LEARNING POINTS

- Growth can be divided into four phases – fetal, infancy, childhood and pubertal.
- *In utero,* insulin and the IGFs control growth, while the impact of GH is limited.
- Later pubertal onset and higher peak growth velocity account for the height difference between males and females.
- Production of GH is influenced by GHRH, somatostatin and ghrelin.
- Two variants of GH circulate bound to GHBP and signal via a transmembrane GHR.
- IGFs circulate bound as part of a ternary complex to an IGFBP and ALS, which prolongs the half-life of the IGFs.
- The diagnosis of GHD is based on assessment of auxology, biochemical assessment of the GH-IGF-1 axis and neuroimaging. In selected cases genetic evaluation may also aid diagnosis.
- Pharmacological stimulation tests display great variability depending on the assay and stimulus used, as well as the presence of obesity and the use of sex steroid priming.
- In GHD recombinant GH therapy is highly efficacious with an excellent safety profile.
- While extensive data exists in animal models linking upregulation of the GH-IGF-I axis to cancer risk, this is not seen in patients treated with rhGH who have no prior history of cancer.
- Poor compliance to GH therapy is common and should be considered where there is a poor response to treatment.
- Integrating current prediction models into clinical practice has been shown to reduce variability in response to treatment but does not improve overall response.
- The commonest tumour of the hypothalamo–pituitary area in childhood is a craniopharyngioma that presents with visual symptoms, endocrinopathy and/or symptoms from raised intracranial pressure.
- GH insensitivity is characterized by poor growth in combination with low IGF-I concentrations and normal or high stimulated GH concentrations.
- The IGF-I generation test has a low specificity.
- Recombinant human IGF-I is significantly less efficacious in treating GH insensitivity than GH treatment in GHD.
- 90% of children born SGA will display catch-up growth by 2 years of age.
- GH is licenced to treat those SGA children who do not show catch up growth.
- An improved response to treatment in short SGA children is seen where therapy is initiated at an earlier age and at a higher starting dose.
- rhGH therapy is associated with a height gain of 7–11 cm in TS.
- Oxandrolone has been shown to improve final height by an extra 1.1–4.6 cm when used in combination with rhGH.
- All patients diagnosed with TS should undergo evaluation for cardiac and renal abnormalities.
- Patients with PWS are often highly sensitive to GH therapy and the use of lower starting doses is recommended.
- Scoliosis is common in PWS and in most cases GH should not be discontinued when this develops.
- Normocephalic/macrocephalic SGA disorders are associated with genetic aetiologies distinct from those causing microcephalic SGA disorders, making this a useful clinical feature.
- Early puberty and adrenarche with bone age advancement may limit the efficacy of GH therapy in SRS.
- Gene mutations found in the ISS population are rare and affect genes expressed in the growth plate previously associated with skeletal dysplasias.
- CNVs can be found in 10–20% of patients with unexplained severe short stature.
- rhGH is licenced in the USA but not in Europe for the treatment of ISS.
- There is currently insufficient evidence to recommend other therapies such as IGF-I, aromatase inhibitors or GnRH analogue therapy in ISS.
- Skeletal dysplasias should be identified by the presence of disproportionate short stature. The first stage of assessment is to determine whether there is truncal or limb shortening and, in the case of limb shortening, which segment is affected.
- In trials, rhGH therapy has a modest impact in children with achondroplasia or hypochondroplasia and is not a licensed indication.
- rhGH therapy is however licensed for patients with SHOX deficiency and efficacy is similar to that seen in Turner syndrome.
- Most children with tall stature will have a benign cause such as familial tall stature or constitutional tall stature.
- Treatment of tall stature is not always required and can be either with the use of sex steroids or surgery (epiphysiodesis).

Brook's Clinical Pediatric Endocrinology, Seventh Edition. Edited by Mehul T. Dattani and Charles G. D. Brook.

Companion website: www.wiley.com/go/dattani/brookcpe7

To understand the processes that lead to normal growth, the patterns of normal growth and the hormonal and growth factors involved, it is critical to identify and define the mechanisms that underpin growth disorders. This chapter presents key aspects of the normal physiology of growth and the aetiology of growth disorders followed by a focus on individual conditions causing short and tall stature.

Normal Growth

Growth is characterized by an increase in size by accretion of tissue mass and is dependent on the balance between cell hypertrophy, cell hyperplasia and apoptosis. Disorders of growth are the commonest reason for referral to a pediatric endocrinologist.

Growth can be divided into fetal, infancy, childhood and pubertal phases (Figure 6.1) and the factors influencing growth in each phase are distinct. With a crown-rump velocity of up to 62 cm/year during the second trimester and 48 cm/year during the third trimester, the fetal phase includes the fastest period of growth during the human lifespan. Increase in fetal weight is also rapid but is higher in the third trimester at 8.7 kg/year compared with the second trimester at 2.7 kg/year.

The main endocrine factors controlling fetal growth are insulin and the insulin-like growth factors IGF-I and IGF-II: cord blood concentrations of each of these correlate with birth size. Placental function and maternal nutrition are the most important non-endocrine factors influencing growth during this period. Growth hormone (GH) plays only a minor role in growth during intrauterine life, as demonstrated by the very small reduction in birth weight in children born with congenital growth hormone deficiency (GHD). This is in stark contrast to neonates born with severe insulin resistance syndromes or IGF-I deficiency, both of which are associated with severe intrauterine growth impairment.

There is a rapid decline in growth velocity during the first year of life from an initial peak velocity of 25 cm/year to around 10 cm/year by the end of the first year. Growth during this period is dependent on nutrition and thyroid hormone. It had been thought that GH did not play a major role during this period but it is now clear that children with GHD display reduced growth velocity from birth.

Between 6 months and 3 years of age, there is a transition from the infancy phase to the childhood phase where growth velocity remains fairly constant at 4–7 cm/year. During the immediate prepubertal years, growth slows with the lowest growth velocity occurring just before the onset of puberty. GH, IGF-I and thyroid hormone are the primary drivers of childhood growth.

Size at birth correlates poorly with parental size but there is a period of catch-up or catch-down growth over the first 2 years and correlation with parental size improves significantly. There are two main theories for the mechanisms underlying catch-up growth. The first, originally proposed by Tanner, is the neuroendocrine hypothesis, which suggests that there is a central process detecting the difference between expected and actual sizes. Where there is a mismatch, growth velocity is increased, presumably through a central mechanism. There is no empirical evidence for this hypothesis. The second hypothesis is that it arises from a delay in growth plate senescence. The proliferative rate of growth plate chondrocytes normally decreases with each successive

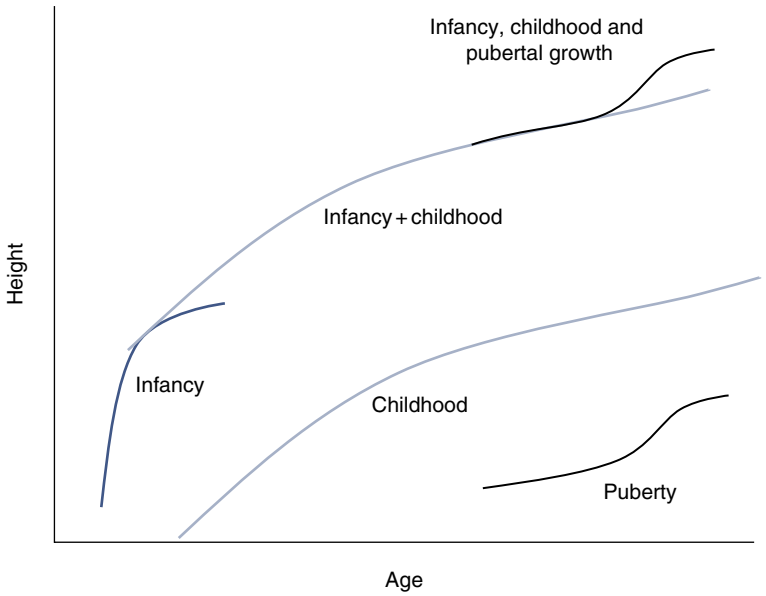

Figure 6.1 The infancy, childhood, puberty model of growth.

cell cycle. Growth delay is associated with a reduced number of stem cell divisions during the period of growth suppression. After cessation of growth suppression, there is a compensatory increase in cell proliferation within the stem cells of the growth plate leading to catch-up growth.

The transition from the prepubertal state with the development of secondary sexual characteristics to final adult height is the last stage. The pubertal growth spurt occurs on average 2 years earlier in females and reaches a peak of 8 cm/year coinciding with the onset of puberty; the peak growth velocity in males reaches 10 cm/year and occurs later in puberty coinciding with a testicular volume of 10–12 mL. The difference in height between males and females is due to the later onset of the pubertal growth spurt in males with the additional years of prepubertal growth and the greater magnitude of the male pubertal growth spurt.

Puberty begins with reactivation of the hypothalamo–pituitary–gonadal axis leading to the production of androgens (in males) and oestrogen (in females). Sex steroid production is associated with an increase in activity of the GH/IGF-I axis. Administration of testosterone to pre- or peri-pubertal boys increases spontaneous and stimulated GH secretion and IGF-1 concentrations by an effect on GHRH that is dependent upon aromatization: co-administration of an oestrogen receptor antagonist or of dihydrotestosterone (the active form of testosterone that cannot be aromatized) does not lead to an increase in GH or IGF-I. IGF-I concentrations and GH secretion also increase during puberty in girls but the mechanisms underlying this are less clear. Administration of oral or transdermal oestrogen induces a decline in serum IGF-I concentrations and a consequent increase in GH secretion.

Growth ceases with fusion of the epiphyseal growth plate that is induced by oestrogen acting on the oestrogen receptor-α (ERα), as evidenced by patients with defects in the aromatase or ERα genes whose growth plates fail to fuse resulting in significantly tall stature.

Physiology of Growth

The GH/IGF-I axis

The secretion of GH from the pituitary is regulated by hypothalamic secretion of growth hormone–releasing hormone (GHRH). GHRH acts to release stored granules of GH as well as upregulating expression of the *GH1* gene. Release of GHRH is pulsatile and under the control of somatostatin and ghrelin. Ghrelin is produced in the stomach and acts via the GH secretagogue receptor to stimulate GH release and secretion of insulin, ACTH and prolactin. The active hormone is the octanoylated form produced by ghrelin O-acetyltransferase and is cleaved from the 117 amino acid preprohormone. It also increases appetite and regulates food intake, adiposity and metabolism. Ghrelin therefore acts as a link between growth and metabolism, although its precise role in human growth still remains unknown.

Preprosomatostatin is produced in the neurons of the anterior periventricular nucleus that project to the median eminence. Somatostatin is derived from cleavage of preprosomatostatin into two main isoforms of 14 and 28 amino acids. Somatostatin acts through a family of five distinct somatostatin receptors (SSTR1-5) with distinct expression patterns. SSTRs 1, 3 and 5 are expressed in the anterior pituitary and activation of these receptors by somatostatin inhibits GH secretion by inhibiting GHRH secretion and the secretion and activity of ghrelin. While GHRH stimulates membrane depolarization by opening sodium channels (leading to hormone secretion), activation of somatostatin receptors leads to opening of potassium channels and membrane hyperpolarization. Somatostatin tone determines trough concentrations of GH and reductions in somatostatin tone are a major factor in determining the timing of a pulse of GH. In addition to the control by somatostatin and ghrelin, GH secretion is stimulated by hypoglycaemia and exercise and inhibited by IGF-I.

The release of GHRH associated with a reduction in somatostatin tone leads to the pulsatile pattern of GH secretion, with the pulses mainly occurring at night. The amplitude of the pulses increases from childhood to a peak in adolescence consistent with the highest growth rate and IGF-I concentrations during the pubertal years. GH secretion decreases with the end of puberty and the start of adulthood.

Sexual dimorphism can be identified in GH secretion. GH pulses in males are of low amplitude in the day and larger at night, while females have less diurnal variation, more frequent pulses and subsequently a higher basal GH production. Trough GH concentrations have been linked to BMI and waist–hip ratio, but not IGF-I concentrations, while peak GH concentrations are related to IGF-I concentrations. Data in rodents are consistent with the observations in humans, with male rats secreting GH with low trough but high peak concentrations, and having higher growth rates than females, who display a higher basal GH but lower pulsatility. The central and peripheral components regulating GH secretion are shown in Figure 6.2.

Growth Hormone and GH Signal Transduction

There are two GH genes on chromosome 17q23.3. Pituitary GH, a single-chain polypeptide consisting of 191 amino acids with a molecular weight of 22 kDa, is derived from the *GH1* gene; a smaller 20 kDa isoform lacking amino acids 32–46 is generated by alternative

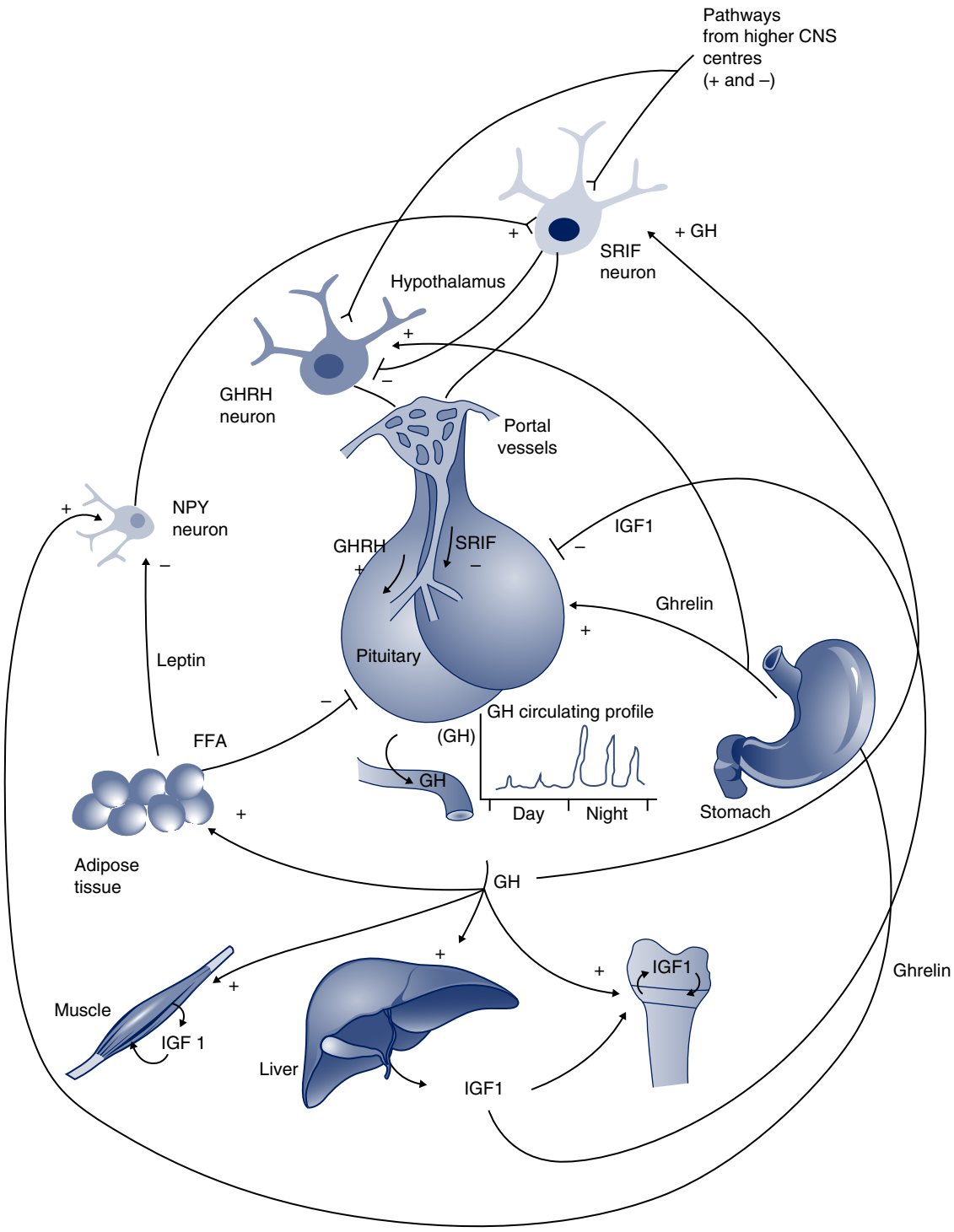

Figure 6.2 Central and peripheral components that regulate the GH axis. NPY, neuropeptide Y; FFA, free fatty acids; GH, growth hormone; IGF1, insulin-like growth factor 1; GHRH, GH-releasing hormone; SRIF, somatotrophin release inhibitory factor. *Source:* Reproduced with permission of Murray et al. [1].

splicing and accounts for 10–20% of circulating GH. The *GH2* gene encodes a 20 kDa variant of GH expressed in placental tissue, but not the pituitary.

GH circulates bound to growth hormone-binding protein (GHBP) that consists of the extracellular domain of the growth hormone receptor (GHR) generated either through proteolytic cleavage of the GHR or by alternative splicing of *GHR*. The 22 kDa isoform of GH has a higher affinity for GHBP than the 20 kDa and placental GH. GHBP has a molecular mass of 60 kDa and prolongs

the half-life of GH from 11 to 80 minutes as well as maintaining the circulating pool of GH by reducing binding to peripheral GHR.

The actions of GH are mediated through the GHR, a transmembrane receptor consisting of a 620 amino acid extracellular domain, a single transmembrane domain of 24 amino acids and a 350 amino acid intracellular domain. At the cell surface, the GHR is present mainly in a dimerized state. The GH molecule contains two binding sites – 'site one' has a high affinity and 'site two' low affinity – with both binding sites interacting with the same region on the GHR. Binding of a single GH molecule to a dimerized GHR induces a conformational change in the GHR with rotation of one GHR subunit. The structural change locks the extracellular receptor interaction domains together and increases the distance between the intracellular domain box 1 motifs. It is this increase in the distance between box 1 motifs that leads to repositioning of Janus kinase 2 (JAK2) and the initiation of GH signal transduction.

JAK2 phosphorylates the signal transducers and activators of transduction (STAT) molecules including STATs 1, 3, 5A and 5B with the major mediator of GH signal transduction being STAT5A/B. These form homo- or heterodimers and translocate to the nucleus. In addition to activating the STATs, JAK2 also phosphorylates SHC, leading to activation of the mitogen-activated protein kinase cascade and the insulin receptor substrates IRS-1, IRS-2 and IRS-3. The IRS molecules activate phosphatidylinositol-3 kinase ultimately leading to GLUT4 translocation to the cell surface. The only JAK2-independent signal transduction pathways downstream of the GHR is by activation of the Src kinase family (activating the MAPK pathway) and phospholipase C-mediated activation of protein kinase C. Protein kinase C stimulates lipogenesis, c-fos expression, and increases intracellular calcium concentrations by activating type 1 calcium channels. The GH signal transduction system is summarized in Figure 6.3.

Downregulation of GH signalling is achieved via several mechanisms. The tyrosine phosphatase SHP-1 binds to and dephosphorylates JAK2 in response to GH. GH stimulation also induces tyrosine phosphorylation of transmembrane glycoprotein signal regulatory protein, SIRPα1, which recruits and enhances phosphorylation of SHP-2. SHP-2 dephosphorylates SIRPα1, Jak2 and the GHR.

The net result of GH signal transduction is the transcription of a set of GH- dependent genes and the production of IGF-I, the combination of which mediates the actions of GH including effects on cell proliferation, bone metabolism, glucose homeostasis and serum lipids.

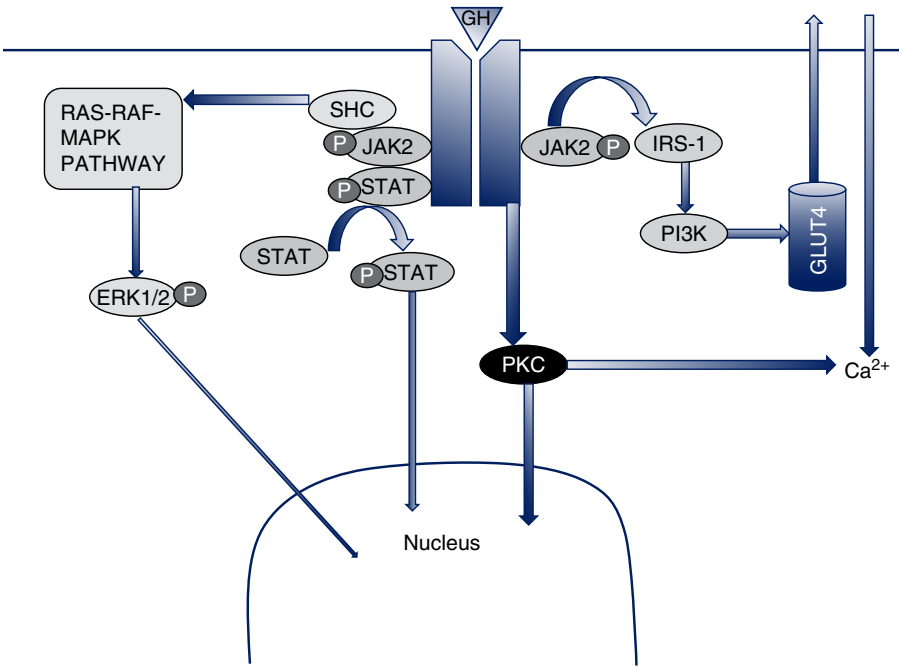

Figure 6.3 GH signal transduction. Binding of GH to two dimerised GH receptors leads to activation of Janus kinase 2 (JAK2), which in turn activates the signal transducer and activator of transcription (STAT) molecules 1, 3 and 5 as well as the RAS-RAF-MAPK cascade and insulin receptor substrate 1 (IRS-1). IRS-1 activation leads to translocation of GLUT4 transporters. JAK2 independent activation of protein kinase C activates calcium channels and increases intracellular calcium.

IGF-I, IGF-II and IGF Signal Transduction

The insulin-like growth factors (IGF-I and IGF-II) are 7.5 kDa single-chain polypeptide hormones sharing 50% homology with insulin. They are produced in the liver and in peripheral tissues and can act in both an autocrine and paracrine manner. IGF-I rather than IGF-II mediates the mitogenic and many of the anabolic effects of GH. IGF-I and IGF-II are both widely expressed with serum concentrations reflecting hepatic IGF production. While IGF-I is regulated by GH, IGF-II is not. Both IGF-I and IGF-II can bind to the insulin receptor as well as the IGF-IR. The IGFs circulate bound to the IGF-binding proteins (IGFBPs), of which there are six classical high affinity IGFBPs. The major IGFBP in serum is IGFBP-3, which is also inducible by GH. The IGFs form a ternary complex with an IGFBP and the acid-labile subunit (ALS), an 85kDa protein secreted by the liver. Formation of the ternary complex is essential to prolong the serum half-life of the IGFs.

The IGF-IR is composed of two extracellular α subunits and two transmembrane β subunits. Ligand-binding sites are in the α subunits, while the β subunits contain three domains: a juxta-membrane domain responsible for recruiting the major signalling proteins; a tyrosine kinase domain, which has an essential role in the catalytic activity of the receptor; and a carboxy terminal domain. Following ligand binding, the IGF-IR recruits and phosphorylates the members of the insulin receptor substrate family of proteins (IRS-1, -2, -3, -4) as well as Shc. Activation of Shc leads to activation of the MAPK pathway, while the IRS proteins activate PI3K via its p85 regulatory subunit leading to activation of AKT, which acts to phosphorylate BAD, inhibiting apoptosis, and to activate mTOR leading to cell survival and growth (see Figure 6.4).

While IGF-I is the predominant ligand for IGF-IR, insulin and IGF-II can also bind, albeit at a lower affinity. Hybrid receptors composed of one insulin receptor α–β subunit and one IGFIR α–β subunit exist in almost all tissues but their biological role remains unclear. Hybrid receptors bind IGF-I and IGF-II with high affinity but insulin with low affinity. The IGF-IIR is also the receptor for mannose-6-phosphate (hence it is also referred to as the mannose-6-phosphate receptor) and is a negative regulator of the growth-promoting actions of IGF-II on the IGF-IR.

Mouse studies have outlined the relative contribution of different components of the GH/IGF axis to growth. Deletion of *Igf1* or *Igf2* results in a birth weight reduction of 40% compared with wild-type mice, while reductions in birth weight of 55% are associated with deletion of *Igf1r*. Combined deletion of *IgfI* +*IgfIr* or *Igf2*+ *IgfIr* leads to a 70% reduction in birth weight and death from respiratory distress at birth, while deletion of *Igf2r* leads to an increase in size to 130% of wild type. It therefore appears that in mouse the IGF-IIR has a role in negatively regulating the availability of IGF-II. From these studies it is clear that up to 70% of mammalian growth may be dependent upon the GH/IGF pathway. Much of the remaining 30% may be accounted for by mechanisms controlling basic cellular processes influencing non-endocrine growth.

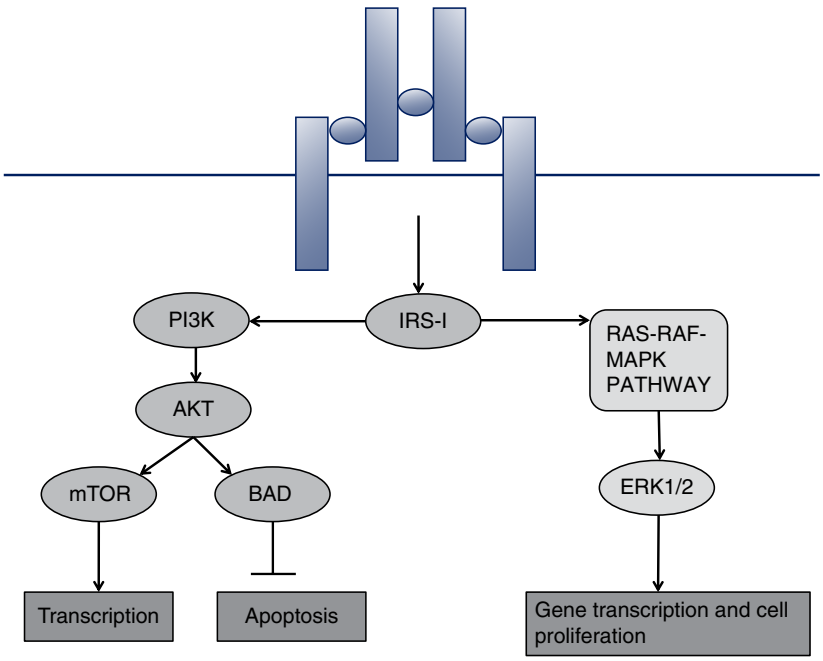

Figure 6.4 IGF-I signal transduction. Binding of IGF-I to the IGF-1R leads to phosphorylation of the receptor and activation of IRS-I. Subsequently IRS-I activates the PI3K, AKT, mTOR and RAS-RAF-MAPK pathways leading to cell proliferation and gene transcription.

Genetic Regulation of Height in the Normal Population

Estimates of the heritability of human height range from 60 to 80% in twin studies. There have been several genome-wide association studies identifying genetic variants (single nucleotide polymorphisms [SNPs]) associated with adult height. The largest contained 253,288 individuals and was able to explain 20% of the variation in adult height from 627 variants clustered in 493 loci. These loci are enriched for genes related to growth pathways as well as genes not previously associated with adult height. It is likely that many children at the lower end of the normal range for stature have inherited multiple genetic variants, each associated with a small reduction in height.

Short Stature

Aetiology of Growth Impairment

There are 10.4 million children and young people in the UK under the age of 16. Short stature is defined as a height >2 SD below the mean, with severe short stature being defined as a height >3 SD below the mean. Depending on the definition 140,000–286,000 children in the UK have short stature for which there are many reasons including:

- Endocrine disorders (e.g. GHD, hypothyroidism, pseudohypoparathyroidism, Cushing syndrome, GH insensitivity including Laron syndrome).
- Chromosomal disorders (e.g. Turner syndrome).
- Systemic disease (e.g. Crohn's disease, juvenile arthritis, coeliac disease, chronic renal failure).
- Constitutional delay of growth/puberty.
- Familial short stature.
- Idiopathic short stature (ISS).
- Born small for gestational age (SGA) with poor postnatal catch-up growth.
- Poor nutrition.
- Neglect (psychosocial deprivation).
- Skeletal dysplasias.
- Syndromic disorders associated with short stature (e.g. Silver–Russell syndrome [SRS], SHOX deficiency, Meier–Gorlin syndrome).

In one classical study the growth of every child between 5 and 10 years was measured in the state of Utah, a total of 114, 881 children. Five hundred and fifty-two (0.5%) with a height less than third centile and height velocity <5 cm/year were investigated and a medical cause for short stature was identified in 74 children (13%). The remaining 87% of short children were given a diagnostic label based on a description of their growth pattern – constitutional delay in growth/puberty, SGA, familial short stature or ISS.

Population-based studies of growth screening have confirmed that only 5–15% of patients with short stature have an identifiable cause of growth impairment with the incidence of pathology rising with increasing severity of short stature. With advances in genetic technologies, a diagnosis may be achieved for some of the children previously diagnosed with ISS or as SGA.

Growth Hormone Deficiency

GHD has a prevalence of ~1 in 4000 during childhood. Although rare, it is an important diagnosis because therapy with recombinant human GH (rhGH) is very effective and a missed diagnosis will result in a poor outcome. The diagnosis includes growth assessment, biochemical investigation of the GH/IGF-I axis and imaging of the hypothalamo–pituitary area. Before evaluation of the GH/IGF-I axis, other diagnoses, such as familial short stature, hypothyroidism, Turner syndrome, coeliac disease, chronic illness such as Crohn's disease and skeletal dysplasias, should be excluded. Consensus guidelines on the diagnosis of GHD in childhood were published in 2000 [2] (https://doi.org/10.1210/jcem.85.11.6984) and suggest investigation in the following scenarios:

1) Severe short stature, defined as a height more than 3 SD below the mean.
2) Height more than 1.5 SD below the mid-parental height.
3) Height more than 2 SD below the mean and a height velocity over 1 year more than 1 SD below the mean for chronological age or a decrease in height SD of more than 0.5 over 1 year in children over 2 years of age.
4) In the absence of short stature, a height velocity more than 2 SD below the mean over 1 year or more than -1.5 SD sustained over 2 years that may occur in GHD presenting in infancy or in organic acquired GHD.
5) Signs indicative of an intracranial lesion.
6) Signs of multiple pituitary hormone deficiency (MPHD).
7) Neonatal symptoms and signs of GHD.

GHD can be isolated (IGHD) or a component of MPHD.

The presentation in the neonatal period is with hypoglycaemia, prolonged conjugated hyperbilirubinaemia and micropenis. Birth size is typically within the normal range, although there may be a reduction of ~10% that occurs typically late in pregnancy. Growth velocity is reduced during the first year of life in children with severe GHD but the phenotype evolves after 1 year of age in those with mild GHD. The earliest manifestations are a reduction in height velocity followed by a reduction in height SDS adjusted for mean parental height SDS. The child's height SDS will ultimately fall below -2SD, the time taken depending on the severity and

duration of GHD. A child with GHD often has midface hypoplasia, hypotonia, a high pitched voice, immature appearance, delayed dentition, thin sparse hair, slow nail growth and truncal adiposity. GHD is also associated with effects on cognition. In mice with GHD, reduced spatial learning and memory has been reported, while in untreated children with GHD IQ, verbal comprehension and processing speed are reduced. In children and adults the neurocognitive defects in GHD are improved by GH therapy.

Biochemical Evaluation of Suspected GH Deficiency

Assessment of GH Secretion

Many assays have been developed to measure GH in serum. A consensus statement recommends that assays should use monoclonal antibodies to measure the 22 kDa variant of human GH and that the reference preparation should be the WHO standard 88/624 (a recombinant 22 kDa human GH at 3 IU = 1 mg) [3]. Significant inter-assay variation exists due to calibration with the different WHO standards, the molecular heterogeneity of GH and the impact of GHBP on assay performance. The coefficient of variation on a GH measurement between assays has been measured as high as 25%, meaning a sample with a mean concentration of 7 µg/L could be reported by different assays with a range between 5 and 10 µg/L. Clinicians should be familiar with the assay used in their local laboratory. Measurement of GH by mass spectroscopy has the potential to circumvent many of these problems as it allows recognition by analyte mass rather than epitope.

During the first week of life, a random measurement of GH <7 µg/L differentiates healthy neonates from those with neonatal GHD. Beyond the neonatal period, measurement of random serum GH concentrations is of no value in the diagnosis of GHD because of the pulsatile nature of GH secretion. This means that physiological/pharmacological stimuli to provoke secretion are required. Consensus guidelines recommend evaluation of the GH/IGF axis by a pharmacological GH stimulation test after exclusion of hypothyroidism, along with the measurement of the downstream GH-dependent factors, IGF-I and IGFBP3. Two GH stimulation tests are recommended, except in those with a history of central nervous system pathology, irradiation or a genetic defect known to cause GHD or MPHD.

Physical and pharmacological stimuli have both been used to provoke GH secretion. Physical stimuli include sleep and exercise but have largely been abandoned because of poor reproducibility. Pharmacological stimuli include insulin, arginine, glucagon, clonidine, pyridostigmine, levodopa and GHRH. The tests can be divided into screening and definitive tests. Tests such as levodopa and clonidine represent screening tests because the agents are administered orally and have relatively low toxicity but the test has low specificity. Definitive tests include the insulin tolerance test, arginine stimulation test and glucagon stimulation test. Stimulation tests rely on a cut-off point used to distinguish GHD from normal GH secretion, but as that is a continuum from severe GHD to normality, any cut-off is arbitrary.

When GH stimulation tests were first used in the 1960s, a peak GH concentration after stimulation of <5 µg/L was used to diagnose GHD on the basis that this concentration seemed to identify patients with a GHD phenotype. Over time this cut-off has increased on the basis of very limited evidence to 7 µg/L and then 10 µg/L in some centres. With the advent of monoclonal antibodies for GH, cut-off concentrations have been set between 4.3 and 7.7 µg/L depending upon the assay used [4]. The study by Wagner et al. [4] represents the best available recent evidence and the cut-offs for each assay derived in that paper can be used where local assay and test-specific data are unavailable.

The insulin tolerance test is considered the gold standard for pharmacological stimulation tests. An intravenous dose of insulin is used to induce hypoglycaemia and the subsequent rise in GH and cortisol as part of the counter-regulatory response is measured through multiple blood samples. After an overnight fast 0.1–0.15 units/kg insulin (reduced to 0.05 units/kg in those under 4 years or 0.1 units/kg where MPHD is likely) is administered and the blood glucose carefully monitored, with a high carbohydrate meal administered after the blood glucose reaches <2.6 mmol/L. Occasionally a bolus of 10% dextrose at 2 mL/kg will also be required to correct hypoglycaemia and intravenous hydrocortisone should be administered for severe or prolonged hypoglycaemia or where there is known adrenal insufficiency. The administration of hyperosmolar solutions of 50% dextrose has been associated with adverse outcomes and is not recommended.

The glucagon stimulation test evaluates GH and cortisol secretion with maximum peak GH concentrations occurring 2–3 hours after glucagon administration. Intravenous and intramuscular glucagon have both been used. However, the glucagon test is a poor test for cortisol secretion. It is thought that the rise in glucose concentrations leads to a rise in insulin secretion that stimulates GH release. Hypoglycaemia can occur as in the insulin tolerance test but less frequently and less predictably. Common side effects include nausea and vomiting. At the end of the test, a meal should be consumed to reduce risk of late hypoglycaemia.

The arginine stimulation test has a lower risk of hypoglycaemia because arginine itself does not cause hypoglycaemia, although fasting for the test may do so in susceptible individuals. An intravenous dose of 0.5 g/kg up to a maximum of 30 g arginine is administered intravenously with subsequent measurement of GH concentrations over 3 hours. Arginine can be used in combination with GHRH, but as this is a particularly powerful stimulus, test- specific peak GH cut-off values in children need to be defined.

Peak GH concentrations reached vary both with the assay used and the pharmacological stimulation test, with higher concentrations reached with the combined stimulation test. Within the normal population, the false positive rate (incorrectly classifying a child as GHD) has ranged from 15 to 50% using a cut-off of 10 μg/L [5] and from 9 to 23% using a cut-off of 7 μg/L. The high false positive rate is the reason for the requirement for a second GH stimulation test in children without additional supporting evidence of GHD such as an abnormality on MRI or known genetic defect associated with GHD.

As an alternative to pharmacological stimulation tests, some units use 12 or 24 hour profiles of GH secretion that requires hospitalization and a blood sample taken every 20 minutes for the time period specified. It is expensive and time consuming. Although the reproducibility of this test is higher than for the pharmacological stimulation tests, it is less sensitive and should be mainly used for the rare diagnosis of GH neurosecretory dysfunction or where pharmacological stimulation tests are contraindicated.

GH neurosecretory dysfunction is defined as an abnormal 24 hour GH secretory profile (reduced number of GH pulses and reduced pulse amplitude) in the presence of a low IGF-I concentration, auxology compatible with a diagnosis of GHD, bone age delay of at least 2 years and a normal GH stimulation test. The condition is most frequently seen after low-dose cranial radiotherapy (<24 Gy), as higher doses reduce both pharmacological as well as spontaneous GH secretion.

Sex Steroid Priming

Children in the peri-pubertal period and those with delayed puberty often display a reduced growth velocity and relative short stature prompting referral for endocrine assessment. Sex steroid production in normal children leads to augmented GH secretion. Therefore, in patients with poor growth and delayed puberty, this augmentation of GH secretion is absent and GH stimulation testing frequently demonstrates low peak GH concentrations. Normalization of the test result has been demonstrated as puberty progresses and most children will retest with normal GH secretion at the end of puberty. This led to the suggestion that the primary pathology was a lack of sex steroids rather than GHD and the recommendation for the use of oestrogen or testosterone priming of GH stimulation tests. Within the normal peri-pubertal population, sex steroid priming reduced the false positive rate from 61 to 5%.

Sex steroid priming for GH stimulation tests remains controversial with surveys suggesting up to 60% of pediatric endocrinologists do not routinely use sex steroid priming in peri-pubertal patients. Currently there are three strategies towards priming taken by pediatric endocrinologists:

1) No priming.
2) Sex steroid priming for any children with pubertal delay (prepubertal at 13–14 years in boys and 11–12 years in girls).
3) Sex steroid priming for all peri-pubertal children (boys >9 years, girls >8 years based on chronological or bone age).

Protocols vary and there is no consensus on the best approach. Common protocols include intramuscular injection of a depot-testosterone 100 mg IM 7–10 days before testing for boys and administration of oral oestrogen (e.g. 10–20 μg ethinylestradiol or 1 mg bd of stilboestrol) for 48–72 hours before testing in both girls and boys.

Obesity

Extensive data indicate that spontaneous and stimulated GH secretion is reduced in obese subjects. With the rising prevalence of obesity, with latest estimates at 14% of UK children, this will have an important impact on evaluation of GH stimulation tests. In adult practice, BMI-specific cut-offs for the diagnosis of GHD have been developed.

Using a conventional (adult) cut-off of 3 μg/L, 45% of normal subjects with a BMI > 25 kg/m^2 tested positive for GHD and a reduced cut-off of 1 μg/L is now suggested for obese subjects. For children undergoing evaluation of the GH/IGF-I axis, a peak GH on stimulation testing of <7 μg/L was present in 50% of patients with BMI > +1 SD compared with 4% in those with BMI between 0 and −1 SD in one report. Within the same group of patients, despite the difference in prevalence of GHD by BMI, there was no difference in serum IGF-I concentrations between groups. Given that classical GHD does present as short stature with increased truncal adiposity, it is perhaps not surprising that the prevalence of GHD rises with increasing BMI. While this may explain part of the increase in the number of subjects classified as GHD at a higher BMI, some of the increase

is likely to be due to false positive tests directly caused by obesity. There is a need for BMI-specific data on peak GH concentrations during pharmacological stimulation testing in childhood.

Assessment of IGF-I and IGFBP-3

Unlike GH, serum concentrations of IGF-I and IGFBP-3 are stable throughout the day. Typically, total IGF-I concentrations in serum are measured via techniques that dissociate IGF-I from its binding proteins. The efficiency of this process or diseases affecting serum IGFBP concentrations, such as diabetes or renal impairment, can influence the total serum IGF-I concentrations. Serum IGF-I concentrations vary with age and pubertal status. In young children, the normal range for IGF-I overlaps with children with GHD and low IGF-I concentrations can also be seen in children with diabetes, hypothyroidism, chronic disease and poor nutrition. During puberty, IGF-I concentrations rise with the sex steroid augmentation of GH secretion so children with delayed puberty can have a low IGF-I concentration for age. IGF-I concentrations defined by pubertal stage or bone age rather than chronological age would be normal in these individuals.

Like serum GH measurements, several reference preparations have also been used for calibration of IGF-I assays. Consensus guidelines now recommend the use of the newer standard WHO IRP 02/254 [3]. Variability of a single measurement of IGF-I is ±35%. A meta-analysis of studies utilizing IGF-I or IGFBP-3 for the diagnosis of GHD identified that a single measurement of IGF-I has moderate sensitivity of 69% (95% CI 63–70%) and specificity of 69% (95% CI 66–72%). In clinical practice, a low or low-normal IGF-I concentration is found in GHD patients while a value of >−1 SD is suggestive of normal GH secretion.

IGFBP-3 was initially suggested to be superior to IGF-I as it is less nutritionally sensitive but multiple studies have since shown that, at best, it offers no benefit over the measurement of IGF-I alone. It is however a useful test in infancy, as the overall higher IGFBP-3 concentrations (in mg/L rather than μg/L for IGF-I) allow better differentiation of normal from low concentrations. In the meta-analysis mentioned above, the sensitivity for IGFBP-3 was 49% (95% CI 45–52%) and specificity was 79% (95% CI 76–82%).

Results from studies combining IGF-I and IGFBP-3 have been variable with some studies suggesting improvement in diagnostic accuracy and others identifying low sensitivity (15% in one study). Combining height velocity with a measurement of IGF-I has been evaluated with the combination having excellent specificity of 96% and sensitivity of 95% but these results require replication in further studies. Another approach has been the assessment of IGF-I/IGFBP-3 ratio that is reported to have higher sensitivity than measurement of IGF-I or IGFBP-3 alone but with a reduction in specificity.

Neuroimaging

The presence of an abnormality within the hypothalamo–pituitary area provides powerful supporting evidence for a diagnosis of GHD. The commonest radiological finding in GHD children is a variable combination of an ectopic posterior pituitary gland, anterior pituitary hypoplasia and a thin or interrupted pituitary stalk. Other abnormalities include tumours such as craniopharyngioma or germinomas, septo-optic dysplasia, corpus callosum hypoplasia/agenesis, holoprosencephaly, thickened pituitary stalk (seen in Langerhans cell histiocytosis [LCH] and germinoma) and the presence of an empty sella. A hypothalamic hamartoma may be seen in Pallister–Hall syndrome. Absent olfactory bulbs or eye abnormalities are seen in patients with genetic defects of forebrain or eye development. GHD may be associated with absence of the internal carotid artery, arachnoid cysts, Arnold–Chiari malformations and syringomyelia.

Genetic Studies

Mutations in *GH1*, *GHRHR* and *RNPC3* have been identified in patients with isolated GHD that may be associated with a normal or small anterior pituitary on MRI scan. The identification of a genetic mutation is particularly useful in supporting the diagnosis in cases of isolated GHD with a normal pituitary MRI. There are many other genes associated with GHD along with other pituitary deficiencies (*POU1F1*, *PROP1*, *LHX3*, *LHX4*, *HESX1*, *OTX2*, *SOX2*, *SOX3*, *GLI2*, *GLI3*, *FGFR1*, *FGF8* and *PROKR2*); there are often other clinical and radiological features in these cases (see Chapter 5).

With the increasing clinical availability of genetic technologies such as whole exome and whole genome sequencing, screening for mutations to provide confirmation of the diagnosis of GHD is likely to increase. An increasing number of patients are being recognized with CNVs (deletions or duplications) of the genes involved in pituitary development. While currently CNV analysis either via array comparative genomic hybridization or SNP array is not routine practice in GHD patients, such tests may have a small but important contribution to make.

Management of Growth Hormone Deficiency

All children with GHD should be treated with rhGH as soon as possible after the diagnosis is made. For most patients therapy will be started after an MRI of the brain and pituitary has excluded the presence of an intracranial

tumour. The primary aim of treatment is to achieve a final adult height within the genetic target range calculated from parental heights.

GH is administered as a once daily subcutaneous injection, usually in the evening. A typical starting dose would be 25–35 μg/kg/day with the dose increased to optimize height velocity and to maintain an IGF-I concentration within the normal range. The usual maximum dose would be ~50 μg/kg/day. Children treated with GH therapy should be reviewed every 3–6 months to optimize dose and check adherence.

Complications that can occur during GH therapy include bleeding/bruising around injection sites, intracranial hypertension, slipped upper femoral epiphyses, scoliosis, insulin insensitivity and hypothyroidism. Intracranial hypertension typically resolves after withdrawal of GH therapy and, after a period off treatment, GH can be reintroduced at a low dose and carefully titrated upwards. The scoliosis and slipped upper femoral epiphyses are related to the increased growth rate seen with treatment. Any minor scoliosis present before therapy will worsen as growth occurs and it is not the GH causing the scoliosis *per se*, but the GH-induced growth. It is very likely that the patient would have developed scoliosis without therapy. Close liaison with a spinal surgeon is important and GH should be stopped only in the most severe cases. Hypothyroidism is common in the population of patients treated with GH because GH decreases TSH secretion and conversion of thyroxine to triiodothyronine. Thus, a patient with marginal thyroid dysfunction may develop central hypothyroidism on GH replacement.

Dose titration is undertaken to optimize growth while keeping serum IGF-I concentrations within the normal range. An alternative strategy has been to titrate the dose of GH based primarily on the IGF-I concentration. Targeting to an IGF-I concentration of 0 SD produced no difference in treatment response compared with a fixed dose. Targeting to an IGF-I concentration of +2 SD did result in an improved first-year height velocity SDS but required an average dose of GH of 90 μg/kg/day, well above the range in which safety data are available. A reasonable combined approach is to optimize growth with an IGF-I concentration in the upper half of the normal range while not exceeding a GH daily dose of 50–60 μg/kg/day.

Another strategy for dosing is to use prediction-based models. The KIGS, Cologne and Göteburg models are three currently available models. Each uses a combination of auxological and biochemical data to predict response to therapy. GH dose is increased where a poor response is predicted and reduced where a good response is predicted. The Göteburg model includes data derived from 12 or 24 hour GH profiles and the Cologne model contains measurement of urinary deoxypyridinoline on GH treatment thus limiting the use of these models to centres where these tests are available. Prediction models reduce variability in response to GH but they do not reduce average GH dose or response to treatment, i.e. the number of patients with a high or low response is reduced but mean change in height velocity is not altered. Key parameters for the KIGS prediction model include peak GH concentration during a stimulation test, age at start of GH therapy, difference between height SDS and mean parental height SDS, GH dose and weight SDS at start of therapy. These explain 61% of the variability in GH response. The only variables the clinician can influence to improve response are the dose of GH (i.e. increasing dose) or the age at start of GH (earlier age predicts better response).

Attempts have been made to predict response to treatment based on genetic factors. The best characterized of these is the exon 3 GHR deletion polymorphism. About 50% of the European population is homo- or heterozygous for this polymorphism. The first report suggested that this deletion increased GH signal transduction and first-year response to therapy in ISS and SGA children treated with GH. There have been many studies examining the effect of this polymorphism in response to GH therapy for all the licenced indications including GHD. A meta-analysis indicates that there is a small size effect increasing first-year height velocity by 0.14 SD in children homozygous for the deletion and by 0.09 SD in children heterozygous for the deletion. The PREDICT study examined the impact of polymorphisms in ~100 candidate genes on change in IGF-I over the first month of therapy and first-year growth response [6]. Ten polymorphisms in 7 of these genes were related to both end points but the effect was small and therefore not sufficiently powerful to develop a predictive algorithm. A genome-wide association approach may yield more useful predictive data but the number of patients required for such a study would be high.

Given the expense of GH treatment, it is essential to identify patients who respond poorly. Age- and sex-specific reference charts for first-year height velocity SDS have been derived from the Kabi International Growth Study and the National Cooperative Growth Study splitting GHD into severe (peak GH <5 μg/L) and less severe (peak GH 5–10 μg/L). A patient with a height velocity SDS of −2 would undoubtedly be defined as a poor responder but this definition is probably too severe. Other definitions of poor response have included increase in height SDS <0.3 or 0.5 SD over 1 year, increase in height velocity <3 cm/year or increase in height velocity SDS <+1 SD. The proportion of children with GHD classified as poor responders varies greatly depending on the definition. Poor adherence (defined as

<85% intended doses administered) is seen in up to two-thirds of patients and evidence of a poor response to treatment should prompt the clinician to examine compliance. If compliance is good, the dose of GH should be increased and IGF-I concentrations monitored. Where response remains very poor despite dose increase, consideration should be given to revisiting the diagnosis and to withdrawing treatment in some cases. It is very rare for children with severe GHD to have no response to treatment.

Safety of Growth Hormone Treatment

rhGH therapy is safe and effective. Before 1985, most patients were treated with pituitary-derived GH. The yield of GH from each pituitary processed was low (~1 mg) so patients were exposed to material from many cadavers and transmission of Creutzfeldt–Jakob disease occurred in 26 of 7700 US patients treated with pituitary GH, a risk not present with rhGH. Unfortunately, more patients were affected in Europe.

Patients with acromegaly have an increased tumour risk and upregulation of the GH-IGF axis increases the number and size of tumours in animal models. Tumours frequently display GH or IGF-I/II receptors on their cell membranes and GH/IGF-I treatment increases growth of cancer cell lines. Epidemiological evidence links higher IGF-I concentrations to increased malignancy risk so the main concern surrounding GH therapy has been the potential for an increased risk of malignancy, particularly since many children treated with GH have a history of brain tumours or other malignancy, such as acute lymphoblastic leukaemia.

Studies of patients treated with GH therapy are generally very reassuring with only modest evidence of a small increase in second malignancies. The Safety and Appropriateness of Growth Hormone Treatment in Europe (SAGhE) study has been collecting data on Europe-wide safety outcomes and mortality in adults treated with GH therapy in childhood. Analysis of the data from France has raised concerns about a small increased mortality risk from both cancer and cardiovascular disease/stroke but an increased risk has not been seen in data from Belgium, the Netherlands and Sweden. The final results of the SAGhE study on cancer risk have now been published. In children with isolated growth failure, overall there was no clear raised cancer risk but there is a small absolute increase in incidence and mortality from bone and bladder cancer [7]. For those individuals treated with GH after a diagnosis of cancer, increased risk of further tumours was associated with increasing daily GH dose but not duration of treatment or cumulative dose.

Reassessment of the GH-IGF Axis at End of Growth

The end of growth can be defined as the time at which growth velocity falls below 2 cm/year. The European Society for Paediatric Endocrinology (ESPE) recommends retesting at the end of growth for all childhood GHD patients, except those with panhypopituitarism (defined as 4 or 5 hormone deficiencies) [8] (http://www.eje-online.org/content/152/2/165.long), while the American Association of Clinical Endocrinologists (AACE) differs and suggests that retesting is not required for patients with genetic mutations causing GHD and structural malformations (tumour, congenital malformation) or those with ≥3 hormone deficiencies [9].

The cut-off for diagnosis of GHD in the transition period is recognized to be lower than the cut-off during childhood. ESPE guidelines suggest a cut-off of 5 μg/L and AACE guidelines propose using the adult cut-off of 3 μg/L. The cut-off based on the most robust evidence is that used in the GH-IGF Research Society guideline of 6 μg/L derived using the insulin tolerance test in young adults. Irrespective of these differing cut-off values, a significant proportion of patients retest as normal with up to 60% of GHD children retesting with a peak GH concentration >10 μg/L, which is normal even using the most liberal cut-off for childhood GHD. Even among those with an ectopic posterior pituitary gland, up to 22% retest with a peak GH concentration >10 μg/L.

When patients are selected for retesting using either the ESPE or AACE criteria, GH therapy should be withdrawn for at least one month. Further evaluation depends upon the likelihood of severe GHD. For those with a high risk (defined as two or three additional hormone deficits, CNS tumour, high-dose cranial irradiation, identified genetic cause or structural abnormality of the hypothalamo–pituitary axis), an IGF-I concentration is evaluated and GH restarted for those with an IGF-I SDS < −2 SD; for these patients a GH stimulation test in the transition period is not required. In the group of young people at high risk of severe GHD, where the IGF-I concentration off treatment is > −2 SD, a GH stimulation test should be performed and GH restarted if this confirms ongoing GHD. Young people deemed to be at a low likelihood of severe GHD (essentially those with isolated idiopathic GHD or those with GHD and only one other hormone deficit) should have an IGF-I concentration and a GH stimulation test evaluated simultaneously with GH restarted if this evaluation confirms ongoing GHD.

The decision to reinstitute GH therapy during the transition period should be made in conjunction with the young person. The accrual of peak muscle and bone mass occurs during this time and GH therapy may optimize this as well as improve fat distribution, cardiac performance and quality of life. A full assessment during the

transition period would include height, weight, body mass index, blood pressure, waist circumference, fasting lipids, bone densitometry and completion of quality of life assessment of GHD in Adults score or other measure of quality of life. Assessing deterioration in these end points may be helpful where the young person declines continuation of GH therapy.

GH should be restarted at a dose of 0.2–0.5 mg/day with dose adjustment based on IGF-I concentrations to take account of the physiological reduction in IGF-I concentrations occurring at the end of puberty.

Monogenic Disorders Causing Growth Hormone Deficiency

IGHD type 1A is an autosomal recessive disorder caused by homozygous or compound heterozygous deletions or nonsense mutations within *GH1*. These lead to complete absence of the GH protein from the serum and severe GHD with growth failure from early life. In common with other patients with severe GHD, the initial response to GH therapy is excellent but the development of antibodies against GH may lead to a loss of treatment efficacy that is unusual in severe GHD. Where patients develop anti-GH antibodies, treatment with recombinant human IGF-I should be considered.

IGHD type 1B is also autosomal recessive and caused by missense mutations in *GH1* or splice site, nonsense, microdeletions or missense mutations within *GHRHR*, the gene encoding the GHRH receptor. Clinical phenotype is milder than in IGHD type 1A with detectable concentrations of GH on stimulation testing. Response to treatment is excellent without the development of anti-GH antibodies. For *GHRHR* mutations, the phenotype is of very low GH concentrations with anterior pituitary hypoplasia on MRI scan. The anterior pituitary hypoplasia is the result of impaired GHRHR action. Midface hypoplasia, neonatal hypoglycaemia and microphallus are less common than among patients with *GH1* mutations.

IGHD type 2 is caused by splice mutations affecting *GH1*, most frequently the first six base pairs of the exon 3 donor splice site. Mutations affecting the exon and intron splice enhancers have also been reported. These mutations lead to exclusion of exon 3 and the production of a 17.5 kDa variant of GH. The variant GH lacks the domain responsible for connecting helix 1 and helix 2 of the mature GH molecule. This leads to retention of the variant within the endoplasmic reticulum ultimately reducing the stability of the 22 kDa GH. Macrophage infiltration of the pituitary gland occurs leading to inflammation and destruction of the pituitary and MPHD.

Type 3 IGHD is an X-linked recessive disorder with GH and immunoglobulin deficiencies. A single patient has been reported with a mutation in the *btk* gene (resulting in exon skipping) with x-linked agammaglobulinaemia and GHD. *SOX3* duplications and mutations are associated with X-linked GHD as well.

Familial isolated GHD has been reported in patients with missense and nonsense mutations in *RNPC3*. *RNPC3* encodes a component of the minor U12-dependent spliceosome responsible for splicing of <0.5% introns in ~3% of human genes. Pituitary hypoplasia is seen on MR imaging. An excellent response to GH therapy without the development of neutralizing antibodies is seen.

There are many genes involved in pituitary development where mutations lead to abnormal pituitary development and a spectrum of pituitary hormone deficits from isolated GHD through to panhypopituitarism. A detailed description of these disorders is given in Chapter 5.

Acquired GH Deficiency

Acquired GHD is less common in pediatric practice than isolated idiopathic or congenital forms. Causes include:

1) Tumours affecting the hypothalamo–pituitary axis, e.g.
   a) Craniopharyngioma
   b) Optic pathway gliomas
   c) Pituitary adenomas
   d) Germinoma
2) Cranial radiotherapy
3) LCH
4) Trauma
5) Lymphocytic hypophysitis

Tumours Affecting the Hypothalamo–Pituitary Axis

Craniopharyngioma

The commonest tumour causing acquired GHD in childhood is a craniopharyngioma, which accounts for 55–90% of sellar and parasellar tumours. They are non-glial embryonal tumours originating either from the ectodermal remnants of Rathke's pouch or residual embryonal epithelium of the anterior pituitary. The incidence is 0.5–2 per million of the population per year with ~40% of cases diagnosed during childhood. In contrast to adult presentation, when most craniopharyngiomas are papillary, the majority in children are adamantinomatous and associated with cyst formation. Survival rates are high with >90% survival 10 years after diagnosis but morbidity is also high from the disease

and from therapeutic interventions. Recently, an activating mutation in *CTNNB1*, encoding beta-catenin in the Wnt signalling pathway, has been identified in the majority of human adamantinomatous craniopharyngiomas, while mutations in *BRAF* have been identified in papillary craniopharyngiomas.

Patients present with symptoms of raised intracranial pressure, visual impairment and symptoms of pituitary hormone insufficiency. Ninety percent of patients have at least one hormone deficiency at presentation, the commonest being GHD. Treatment is with a combination of surgery, radiotherapy and occasionally chemotherapy. Transcranial and transsphenoidal approaches can both be used. Where complete resection can be achieved without damage to the optic nerves and hypothalamus, this is the treatment of choice but the benefits of achieving complete resection in terms of progression or recurrence can be outweighed by surgical morbidity including hypothalamic obesity, diabetes insipidus and blindness. For tumours where complete resection cannot be achieved without substantial risk of such morbidity, more limited surgery should be undertaken with postoperative radiotherapy. Recurrence rates of 20% are comparable to those of complete resection but with lower rates of surgical morbidity. The radiotherapy can be delivered as conventional or proton beam therapy. In theory, proton beam therapy should result in fewer adverse effects as a more limited portion of the brain is exposed to the radiation but this has yet to be proven for pediatric patients with craniopharyngioma.

GH treatment is thought to be safe and effective in craniopharyngioma patients without increased risk of recurrence. Height gain is similar to that seen in congenital GHD. In the presence of confirmed GHD, GH treatment is usually started once tumour treatment is complete and radiological appearance is stable.

Optic Pathway Gliomas

These tumours of the precortical visual pathway may also involve the hypothalamus and present with ophthalmological findings. The commonest associated endocrinopathy is precocious puberty. Active treatment of the tumour is not always required and there is limited or no progression on follow up. Where the tumour progresses, treatment is with radiotherapy or chemotherapy. Chemotherapy is the preferred initial treatment with radiotherapy reserved for patients who have failed to respond to it. GHD and other pituitary hormone deficiencies can be due to the tumour but are more commonly present post-radiotherapy.

Pituitary Adenomas

These tumours can be classified into functional and non-functional adenomas. The former are more common than the latter in children. With increasing resolution from MRI scans and increasing numbers of children undergoing MR imaging, this balance may shift as more incidental small non-functional adenomas are identified. Functional adenomas are most commonly prolactinomas (80%) with ACTH-secreting adenomas (15%) the next most common. GH- and TSH-secreting tumours are extremely rare in childhood. Macroadenomas are associated with GHD but microadenomas are not typically associated with endocrine abnormalities, other than those caused by hypersecretion. Pituitary adenomas in childhood may be caused by mutations in the gene encoding the tumour suppressor menin as well as the gene encoding the aryl hydrocarbon receptor-interacting protein (*AIP*). Recent studies have also implicated microduplications of Xq26.3, including the gene encoding orphan G-protein-coupled receptor *GPR101*, in the aetiology of pituitary gigantism.

Radiotherapy

GHD is the commonest endocrinopathy in patients who have received radiation to the hypothalamo–pituitary axis. The risk is related to the degree of radiation, fraction size and time between fractions. The endocrinopathies caused by radiation exposure evolve over time. For children treated with 27–32 Gy for brain tumours, 50% will be GHD one year after treatment, 85% GHD after 5 years and nearly all after 9 years. As an illustration of dose effect, by 5 years post-radiotherapy, almost all children exposed to >30 Gy cranial radiation will have GHD compared with 65% exposed to <30 Gy. Although risk falls with reducing radiation exposure, isolated GHD has been reported in children exposed to 18–24 Gy used for prophylaxis in acute lymphoblastic leukaemia and in children exposed to just 10 Gy as part of total body irradiation.

Increased dose does not increase the risk of just GHD but also increases the risk of MPHD: the 18–30 Gy dose used in the treatment of leukaemia leads to isolated GHD and 60 Gy used to treat skull base tumours leads to MPHD. Within the HP axis, there is a hierarchy of sensitivity to radiation-induced damage with the GH axis affected first, followed by GnRH-LH/FSH, CRH-ACTH and TRH-TSH axes.

The site of damage after radiotherapy appears to be the hypothalamus as modest doses of radiotherapy <50 Gy affecting the hypothalamus result in >90% of patients being affected by hormone deficiencies, while much larger doses of radiation delivered to the pituitary alone result in lower rates of endocrinopathy (40% of subjects affected by endocrinopathy at 14 years post-exposure). Some children, particularly those with brain tumours with the capacity for exfoliating cells into the CSF (e.g. medulloblastoma and ependymoma), are treated with radiotherapy to the spine as well as the brain. This spinal radiotherapy

has a profound effect upon growth and children receiving this treatment reach a significantly lower height SDS as adults (~1–1.5 SD lower) than those who received cranial radiotherapy alone.

Efficacy of GH therapy in children treated with radiotherapy is generally poorer than children treated for congenital GHD. In congenital GHD, significant catch-up growth is generally seen but in children receiving cranial radiotherapy GH largely prevents a further decline in height SDS but catch-up growth may not be seen. Children who received craniospinal radiotherapy respond even less well to GH therapy with poor growth of the spine and relatively preserved long bone growth leading to disproportion. The poorer response to GH therapy is likely to be due to the direct effects of radiotherapy on the spine, early puberty, delay in starting GH therapy while the child is undergoing tumour-directed therapies and the use of lower doses of GH in this patient group.

IGF-I and IGFBP-3 concentrations are frequently normal in children with radiation-induced GHD, making the diagnosis more challenging. This is especially the case in radiation-induced neurosecretory dysfunction where pharmacological stimulation tests will be associated with normal GH concentrations.

Langerhans Cell Histiocytosis

Abnormal proliferation of clonal dendritic cells bearing an immune phenotype similar to the Langerhans cells of the skin is seen in LCH. These abnormal cells can spread to nearly any site within the body, proliferate and lead to a local inflammatory response. LCH is a rare disorder affecting ~4 per million children. The commonest endocrinopathy is diabetes insipidus with GHD in about 10% of patients.

The patient may present with disease outside the pituitary and later manifest diabetes insipidus *with or without* growth impairment. The diagnosis is more challenging when the patient presents with a pituitary mass or pituitary stalk thickening without extra-pituitary manifestations of the disease. In these patients a careful search for extra-pituitary lesions is important as alternative sites affected may be easier to biopsy to achieve a histological diagnosis. Pituitary/pituitary stalk biopsy requires an experienced pituitary surgeon but reports suggest a low incidence of additional post-operative hormone deficiencies. Involvement of an oncologist or other clinician experienced in treating these patients is vital.

Trauma

The literature on trauma and hypopituitarism has expanded significantly. Traumatic brain injury (TBI) is common in childhood and injuries to the hypophyseal vessels (through shearing forces during violent impacts) leading to anterior pituitary ischaemia are hypothesized to lead to hypopituitarism. An increased risk of hypopituitarism has been demonstrated in adults after TBI with GH and gonadotrophin deficiencies being the most common. The literature on childhood TBI as a cause of hypopituitarism is less convincing. One of the largest studies to date examined 198 survivors of childhood TBI and identified a low peak GH concentration in 16, but all of these subjects had a height SDS and IGF-I concentration within the normal range. Several smaller studies have identified a high rate of relatively minor, clinically insignificant abnormalities. Current evidence does not support routine surveillance of pituitary function in childhood TBI patients but clinical evidence of an endocrinopathy (e.g. poor growth) should signal the need for investigation.

Hypophysitis

Hypophysitis, inflammation of the pituitary, can occur as lymphocytic, xanthomatous, granulomatous, necrotising, IgG4-related and mixed forms. Lymphocytic hypophysitis is the commonest form but this remains an exceptionally rare disease. Visual problems, headache and vomiting are the common presentations with a homogeneous pituitary mass identified on MRI. This may regress but can also enlarge and affect the cavernous sinuses. The diagnosis requires histological confirmation. While the disorder is more common in pregnant women, men, children and non-pregnant women can also be affected. Surgery may be required if the optic chiasm is compromised. Medical therapy is with immunosuppression (methotrexate, azathioprine, high-dose glucocorticoids), and radiotherapy is also effective.

Disorders of GH Action and GH Sensitivity (Primary IGF-I Deficiency)

Bioinactive GH

Short stature associated with high concentrations of GH and low IGF-I concentrations, described as 'bioinactive GH', was first described in 1978. It is distinguished from GH resistance/primary IGF-I deficiency by a good response to human GH therapy. While there have been many case reports of the condition, few have been associated with a defined genetic aetiology. In some cases mutations of the GH gene (*GH-1*) have been described, e.g. p.G112D, in which functional studies have demonstrated reduced binding of the mutant GH to the GHR. There are other reported mutations such as the p.R77C variant in which functional studies have been unable to identify any deleterious consequences. In short slowly growing children, the combination of normal or high GH concentrations with low IGF-I concentrations is common

(defined as ISS). Molecular investigations are not routinely undertaken. A diagnosis of bioinactive GH should be made only when functional studies have demonstrated adverse effects of a *GH-1* mutation and when there has been a clinical response to GH therapy.

Laron Syndrome

Missense, nonsense, insertions, deletions and splice mutations within the *GHR* gene have been described in patients with the clinical phenotype of GHD (frontal bossing, midface hypoplasia, growth failure, normal intelligence) but with high GH concentrations and low IGF-I concentrations. ALS and IGFBP-3 concentrations are also low. Untreated adult height is around −5 SD. Measurement of GHBP in serum is useful as GHBP represents the extracellular domain of the GHR and its absence or markedly reduced concentrations of GHBP are consistent with the diagnosis of Laron syndrome.

The presence of GHBP in serum, however, does not exclude a diagnosis of Laron syndrome as mutations affecting the transmembrane domain, intracellular domain or missense mutations may not cause loss of the protein but can still impair function of the GHR. The standard diagnostic test has been the IGF-I generation test. There are several protocols for this but one example would be the administration of 0.025 mg/kg/day GH for 5 days with IGF-I measured before the first injection and 12 hours after the last injection. A rise in IGF-I concentration after GH > 15 µg/L has been suggested as excluding GH insensitivity. Using these diagnostic criteria the specificity of the IGF-I generation test is reported to be only 77–91%. Thus when this test is applied to a population with a low prevalence of Laron syndrome, the positive predictive value is very low. The diagnosis of Laron syndrome therefore relies on an integration of clinical, biochemical and genetic information. Sequencing of the *GHR* gene is useful in confirming the diagnosis.

Where there is a family history of Laron syndrome and a known mutation within the family, rapid screening for this mutation can confirm the diagnosis without the need for any dynamic endocrine studies. An experienced laboratory is vital in analysing the results of sequencing, in particular screening for known intronic variants such as those causing pseudoexon activation, which would be missed through standard whole exome sequencing techniques.

STAT5B Deficiency

STAT5b is a major component of the downstream signal transduction cascade following activation of the GHR. Human mutations of STAT1 and STAT3 are associated with immune disease while the initial report of a human STAT5b mutation was in a patient with both GH insensitivity and an immune deficiency. This report was of a missense mutation affecting the SH2 domain leading to aberrant folding and aggregation of the protein. Among the small number of patients reported subsequently, a further SH2 mutation was identified along with a number of nonsense and frameshift mutations.

The major clinical feature differentiating STAT5b deficiency patients from those with Laron syndrome is the immunodeficiency that presents as chronic or recurrent pulmonary infections and lymphoid interstitial pneumonia. Haemorrhagic varicella has also been reported. The respiratory disease is severe with half of the patients dying from their lung disease or requiring a lung transplant. Prolactin concentrations are raised in STAT5b but not Laron syndrome patients. The growth impairment is similar to that of Laron syndrome and facial phenotype also includes a prominent forehead and depressed nasal bridge. The biochemical findings are similar to Laron syndrome with raised basal and stimulated GH concentrations and low IGF-I, IGFBP-3 and ALS concentrations before and after GH treatment as part of an IGF-I stimulation test.

Acid-Labile Subunit (ALS) Deficiency

Deficiency of ALS presents with less severe growth impairment and delayed puberty. Birth weight is almost always within the lower half of the normal range with a normal birth length. Postnatal growth impairment affects the majority of individuals with biallelic *ALS* mutations, with prepubertal height ranging from −4 to −1 SD. Mean untreated final adult height is ∼ −2 SD. Pubertal onset is delayed in 80% of males. Serum concentrations of IGF-I and IGFBP-3 are extremely low (from −3 to −18 SD) and ALS concentrations usually undetectable. A picture of GH insensitivity is seen with a raised peak GH on pharmacological stimulation testing.

A diagnosis of *ALS* deficiency should be considered where there is a biochemical picture of GH insensitivity with a milder growth impairment and pubertal delay. Although not commonly available, measurement of ALS concentrations would be useful to distinguish ALS deficiency from ISS in which there can be evidence of a milder insensitivity within the GH-IGF axis with low IGF-I concentrations and raised stimulated plasma GH concentrations.

ALS deficiency is an autosomal recessive disorder with a variety of homozygous and compound heterozygous mutations identified including missense, nonsense, deletions, duplications and insertions. The central 20 leucine-rich domains contain most of the mutations. The relatively mild growth impairment seen in ALS deficiency is in stark contrast to the other inherited GH-IGF-I axis disorders. The explanation for this is likely to

be that in ALS deficiency there is a loss of the hepatic-derived circulating IGF-I/IGFBP-3/ALS ternary complex but local IGF-I production and action in the bone may be less affected. This is in keeping with mouse data where liver-specific deletion of *Igf1* leads to very modest growth impairment, while whole-body *Igf1* deletion (i.e. both hepatic and locally derived IGF-I removed) leads to profound growth impairment.

IGF-I Gene Deletions and Bioinactive IGF-I

The first patient with an IGF-I deletion was described in 1996 with only four further patients described since then. The clinical presentation is with severe pre- and postnatal growth restriction with sensorineural deafness, microcephaly and developmental delay. Serum concentrations of IGF-I are extremely low or undetectable with normal concentrations of IGFBP-3 and ALS. Both baseline and stimulated concentrations of serum GH are raised. The first patient had a homozygous deletion of exons 3 and 5 of *IGF1* with subsequent reports of missense mutations, two of which have been shown to affect binding of IGF-I to the IGF1 receptor – this variant can be termed bioinactive IGF-I. A splice site mutation affecting exon 4 has also been reported associated with a less severe phenotype with normal birth size, normal intellect, postnatal growth restriction, mildly reduced IGF-I concentrations and attention-deficit hyperactivity disorder. There were individuals with short stature, but without the splice site mutation, within the same family, and thus the pathogenicity of this mutation remains unclear.

The classical picture of IGF-I deletions/bioinactive IGF-I is easy to distinguish from other GH insensitivity disorders by the presence of intellectual impairment, hearing loss, prenatal growth impairment and normal IGFBP-3 concentrations. Treatment is with recombinant human IGF-I that can be complicated by the development of antibodies to IGF-I in those with *IGF1* deletions.

Recombinant Human IGF-I Therapy

Recombinant human IGF-I therapy is licenced for the treatment of primary IGF-I deficiency in children meeting the following criteria:

1) Height SDS < –3 SD.
2) IGF-I concentrations <2.5th centile for age and gender.
3) GH sufficiency.
4) Exclusion of secondary forms of IGF-1 deficiency, such as malnutrition, hypothyroidism or chronic treatment with pharmacologic doses of anti-inflammatory steroids.

The most extensive data on using recombinant human IGF-I to treat primary IGF-I deficiency are in children with Laron syndrome where the results of treatment of 28 individuals for 5 years with 120 µg/kg/day of recombinant human IGF-I improved height SDS from –6.1 to –5.1 SD. First-year height velocity showed a marked increase but this improvement rapidly disappeared after the first year of treatment. Better results have been seen with greater duration of treatment: 21 patients with a diagnosis of GH insensitivity (5 of whom had confirmed Laron syndrome) were treated for a mean of 10.5 years with height SDS increasing by 1.9 SD.

There is a marked difference between the highly efficacious GH therapy in GHD and the much less effective therapy with IGF-I in primary IGF-I deficiency. This is explained partly by the combined effects of GH on hepatic and local production of IGF-I within the growth plate, in contrast to IGF-I injections where this is predominantly replacing the circulating IGF-I rather than IGF-I generated in the growth plate. In addition, for most of the primary IGF-I deficiency disorders, there are also low concentrations of serum IGFBP-3 and ALS; without these the half-life of IGF-I is markedly reduced.

Recombinant IGF-I therapy is contraindicated in those with active malignancy or a known acute hypersensitivity reaction to IGF-I. The starting dose is 40 µg/kg twice daily that is increased over ~3 months to the therapeutic dose of 120 µg/kg twice daily. The main immediate complication of therapy is the potential for hypoglycaemia; IGF-I should therefore be administered after a snack or meal. Some children treated with IGF-I are already at an increased risk for hypoglycaemia, e.g. children with Laron syndrome. A short hospital admission should be considered when initiating therapy and the parents trained in measuring capillary blood glucose and the correction of hypoglycaemia should it occur. It is recommended to avoid strenuous activity immediately after the medication is administered during the initiation phase. Hypertrophy of lymphoid tissue has been noted in patients receiving IGF-I treatment. Where there are symptoms of sleep apnoea or snoring before or during treatment, consideration for ENT assessment and sleep study should be made. In some patients coarsening of facial features has been noticed. It may be useful to take baseline clinical photographs before initiating therapy.

Side effects of IGF-I in common with GH therapy include intracranial hypertension and slipped upper femoral epiphyses. Local and systemic allergic reactions may occur and antibodies to IGF-I may develop, potentially compromising treatment efficacy. Lipohypertrophy may occur when injection sites are not rotated regularly.

Children on IGF-I therapy should be monitored every 3–4 months with assessment of auxology, blood pressure, occurrence of hypoglycaemia and symptoms of sleep

apnoea and monitoring of injection sites. Monitoring of IGF-I concentrations is not recommended.

Disorders of IGF-I Resistance

Mutations within *IGF1R* give rise to a clinical phenotype similar to but less severe than patients with deletions affecting *IGF1*, in that they are born SGA and show postnatal growth impairment with subsequent short stature. Affected individuals also share the microcephaly and developmental delay. The head circumference and birth weight are approximately equally impaired with SDS −1.5 to −3.5; birth length is more variable and may be extremely short with an SDS up to −5.0. The biochemical phenotype distinguishes patients with an *IGF1R* mutation from those with an *IGF* deletion as the IGF-I concentrations are normal or raised. For the reported individuals with an *IGF1R* mutation and a normal IGF-I concentration, the IGF-I concentration is almost always > +1SD.

A wide variety of missense and nonsense mutations, deletions and duplications have been identified leading to impaired function of the *IGF1R* through a variety of mechanisms including nonsense mediated decay, production of a truncated protein, altered protein trafficking and reduced ligand binding activity. To date, the majority of patients have heterozygous mutations and the condition is inherited in an autosomal dominant fashion.

Abnormalities of chromosome 15 including ring chromosome, monosomy and unbalanced translocations result in impaired growth. *IGF1R* is located at chromosome 15q26 and this has led to the hypothesis that the growth impairment in these patients is due to IGF-I resistance. The clinical phenotype is variable and dependent upon the underlying chromosomal abnormality.

Treatment of IGF insensitivity is challenging. Many patients with *IGF1R* mutations do not respond to treatment with rhGH. Theoretically it may be possible to overcome partial IGF-I resistance by generating supraphysiological concentrations of IGF-I. Typically, when treating growth disorders, the aim of treatment is to maximize growth without generating such supraphysiological concentrations of IGF-I. The risks of long-term high concentrations of IGF-I are unclear. It may be that patients with an *IGF1R* mutation will also be resistant to the adverse effects of high IGF-I concentrations, but this is unknown. A partial response has been seen in some patients, with the best response seen in a patient treated with a modest GH dose (25 µg/kg/day). A careful discussion of the paucity of information on risks of using GH in this group of patients should be undertaken before initiating therapy. Response to GH therapy appears to be better for those with chromosome 15 abnormalities in comparison to those with *IGF1R* mutations, with a first-year increase in height SDS of 0.5–1.5 SD.

Growth Disorders not Related to the GH-IGF Axis

Growth disorders may be caused by a range of aetiologies and/or associated with specific circumstances (e.g. being born SGA). Many of these disorders are not primarily related to abnormalities within the GH-IGF axis, although dysfunction in the axis may be found (e.g. GHD in Prader–Willi syndrome).

The Short Small-for-Gestational-Age Child

Multiple definitions of SGA have been used including a crown-heel length less than the 10th, 5th or 3rd centiles. Definitions commonly used in clinical practice include a weight <10th centile, most commonly used in neonatal medicine, or a birth weight or length more than 2 SD below mean, as recommended by pediatric endocrine consensus guidelines [10] (https://doi.org/10.1210/jc.2006-2017). Although birth length can be used to define SGA, there are many countries in which this measurement is not routinely undertaken. By definition 2.3% of the normal population will be SGA using a definition of birth weight < −2 SD but these population studies exclude individuals affected by significant disease processes and the true incidence of being born SGA has been measured at 3% for birth weight < −2 SD and 3.9% for a birth length < −2 SD. 1.5% of newborns have both weight and length SDS < −2 at birth.

Ninety percent of all children born SGA experience catch-up growth within the first two years of life, although this can be slower in children who were also born preterm in whom catch-up growth may continue up to 4 years of age. Despite the vast majority of children born SGA experiencing catch-up growth, final height for this group is still 1 SD below the population mean.

SGA is distinct from intrauterine growth restriction (IUGR), which refers to the situation in which growth restriction occurs *in utero*; while it is possible to be born SGA having experienced IUGR, it is also possible to be exposed to a period of IUGR without being born SGA (e.g. a late gestation fetus may suffer IUGR but may be born within the normal range for birth weight). Equally a constitutionally small fetus may not suffer from IUGR yet be born SGA.

In the neonatal period SGA is associated with increased risk of hypotension, hypoglycaemia, necrotizing enterocolitis and death. In later childhood, children born SGA have lower cognitive performance than appropriate-for-gestational-age children and have a higher risk of

hypertension, type 2 diabetes, hyperlipidaemia and cardiovascular disease in adult life.

Causes for a child to be born SGA include maternal, placental and fetal causes:

- Maternal – poor nutrition, substance abuse, smoking, infections, medical conditions.
- Placental – insufficiency, abruption, infarction, structural anomalies.
- Fetal – congenital infections, chromosomal malformations, monogenic disorders (e.g. 3-M syndrome, Mulibrey nanism, Bloom syndrome).

Management of Child Born SGA

Children born SGA should have their weight, length and head circumference measured every 3 months for the first year of life and 6 monthly thereafter. Individuals who do not manifest significant catch-up growth in the first 6 months of life or those who remain short by 2 years of age may have other conditions that limit growth and additional investigations appropriate to the clinical findings should be pursued. Rapid weight gain in the first 6 months of life is associated with increased cardiometabolic risks in later life. Breast feeding reduces the risk of obesity and should be promoted in SGA infants. Given the association between rapid weight gain and disease risk in adulthood, caution should be exercised in using calorie-dense feeding.

GH is licensed both in Europe and the USA for treatment of the SGA infant with failed catch-up growth. In the USA, treatment is licensed from 2 years of age and the recommended dose is 70 μg/kg/day. The European licence is for treatment with a starting dose of 35 μg/kg/day with the following criteria:

1) Age > 4 years.
2) Birth weight <−2SDS.
3) Height SDS < −2.5 SD compared with population reference data and more than 1 SD below mid-parental height SDS.
4) Growth velocity SDS < 0 SD.

Factors associated with an improved response include age at start (lower age associated with better response), height SDS at start of treatment (lower height SDS associated with better response), mid-parental height and starting dose. Clinicians should start GH treatment at the earliest opportunity and use the highest dose within the recommended range of 35–70 μg/kg/day that is not associated with the development of supraphysiological IGF-I concentrations.

In common with other growth disorders, much of the height gained is during the first 2 years of treatment, but treatment should not be discontinued as this can lead to catch-down growth. In children who do not display a response to GH therapy after 1 year, treatment should be discontinued. In those showing a response, treatment should be continued until growth velocity is <2 cm/year. End of growth assessment of the GH-IGF-I axis is not required.

Although puberty in the majority of children born SGA occurs within normal limits, there is some evidence of menarche 5–10 months earlier in girls born SGA. GnRH analogue therapy can be used to suppress puberty where there is true precocious puberty. In cases where the onset of puberty is at the lower end of the normal range, the use of GnRH analogue treatment remains controversial. There is, however, increasing evidence that such therapy may improve final height that must be balanced against the psychosocial effects of delaying pubertal progress.

Fasting insulin and glucose should be measured along with thyroid function and an IGF-I concentration before starting treatment. GH therapy is associated with an initial improvement in blood pressure, body composition and lipid concentrations during treatment. However by the end of treatment blood pressure, lipids and body composition are similar to patients who did not receive GH therapy. Adverse events during treatment are not more common in SGA patients treated with GH compared with GHD patients. Thus there is no requirement for routine monitoring of glucose, lipids or body composition during treatment.

Turner Syndrome

Turner syndrome affects 1 in 2500 live-born females. The diagnosis requires the presence of characteristic physical features in phenotypic females with complete or partial absence of the second sex chromosome with or without cell line mosaicism. Those with ring X and Xq isochromosomes often present with features identical to those with monosomy X, while those with distal Xp deletions present with short stature (due to *SHOX* deficiency) without other features of Turner syndrome and are at low risk of ovarian insufficiency. The latter should not be diagnosed with Turner syndrome if Xp22.3 is not deleted. Males may have a mosaic Turner syndrome cell line but they are not assigned a diagnostic label of Turner syndrome.

The diagnosis of Turner syndrome should be considered in any girl with unexplained short stature, delayed puberty or any constellation of the clinical findings listed in Table 6.1.

The diagnosis is increasingly being made in the prenatal period because of ultrasound detection of cardiac abnormalities, cystic hygromas or increased nuchal fold thickness prompting amniocentesis and karyotype

Table 6.1 Clinical features of Turner syndrome.

Oedema of the hands or feet
Nuchal folds
Cardiac anomalies especially coarctation of the aorta or hypoplastic left heart
Low hairline
Low-set ears
Small mandible
Short stature with growth velocity <10th centile for age
Markedly elevated concentrations of FSH
Cubitus valgus
Nail hypoplasia
Hyperconvex uplifted nails
Multiple pigmented nevi
Characteristic facies
High-arched palate
Short fourth metacarpal
Chronic otitis media

analysis. There is a high rate of spontaneous termination in Turner syndrome fetuses. Counselling is required by a clinician experienced in the care of Turner syndrome to reflect the variability in its clinical spectrum.

The gold standard for the diagnosis of Turner syndrome in postnatal life is a karyotype. Genetic laboratories are increasingly using DNA microarray (SNP or CGH array) for the diagnosis of CNVs. There have been concerns that some microarray technology may not be able to detect low concentration mosaicism that has led to the recommendation to retain the use of a karyotype for diagnosis of Turner syndrome. With advances in technology and improved resolution of microarray, it is likely that this technology will eventually replace the karyotype. Any karyotype with presence of sex chromosome material of unknown origin should be tested for the presence of Y chromosome material because its presence carries a 12% risk of gonadoblastoma and would justify prophylactic gonadectomy.

Cardiac abnormalities, particularly coarctation of the aorta in 11%, bicuspid aortic valve in 16% and aortic dissection, are associated with Turner syndrome. The prevalence of coarctation and bicuspid aortic valve is increased in patients with neck webbing. Hypertension affects 25% of patients and increases the risk of aortic dissection. All patients should have a cardiac assessment at diagnosis, which would usually involve echocardiography and an electrocardiogram. MRI is more sensitive for the detection of cardiac abnormalities than echocardiogram and recommended once the child is old enough to have this procedure without sedation or general anaesthesia. Urinary system abnormalities are also more common (30–40% of patients), especially collecting system abnormalities and horseshoe kidney. All patients should have a renal tract ultrasound performed at diagnosis.

There is an increased risk of autoimmune disorder with 25% of patients developing hypothyroidism and 5% developing coeliac disease. Screening from the age of 4 years with annual testing for thyroid dysfunction and 2–4 yearly assessment for coeliac disease is recommended. Hearing impairment and middle ear dysfunction are more common in Turner syndrome with a high prevalence of otitis media. Any girl with Turner syndrome who develops otitis media should be referred to an ENT specialist if the effusion fails to resolve. Referral to an audiologist is warranted where patients present late with a history of middle ear problems.

Lymphoedema is a common problem in the neonatal period and infancy but usually resolves before 2 years of age. Diuretics are not helpful and not recommended due to risk of electrolyte imbalance. Involvement of a nurse specialist in lymphoedema is recommended with the mainstay of treatment being support stockings, elevation and decongestive physiotherapy.

The growth pattern in TS is characterized by mild intrauterine growth retardation, slow growth during infancy, delayed onset of the childhood component of growth, slow growth during childhood and the absence of a pubertal growth spurt. GH therapy is licenced for the treatment of short stature in Turner syndrome. After 5.7 years of treatment, GH is reported to increase height by 11 cm more than historical untreated controls. A randomized controlled trial using 0.3 mg/kg/week GH in TS patients aged 7–13 years reported an increase in final height of 7.2 cm in the GH-treated group with a final height of 149 cm [11]. Final height SDS was reported as 0.9–1.3 SDS greater than untreated controls. Higher doses are required than in GHD with doses of 45–50 µg/kg/day recommended. Improved final height is associated with greater parental heights, greater height SDS at start of treatment, higher GH dose, earlier initiation of therapy and longer duration of therapy. Treatment should therefore be initiated as soon as growth impairment becomes clinically apparent.

Oxandrolone has also been demonstrated to improve growth in patients with Turner syndrome. After 4–6 years of therapy oxandrolone was found to increase height by 1.1–4.2 cm [12]. The typical dose would be 0.05 mg/kg/day with a maximum dose of 2.5 mg. The main side effects relate to virilization with clitoromegaly, deepening of voice and hirsutism. Other side effects include hepatic dysfunction, hypertension and deceleration of breast development. Timing of pubertal induction will also influence height gained as delaying pubertal

induction will allow a longer period of prepubertal growth. Too great an emphasis on stature, however, ignores the important psychosocial effects of going through puberty at the same time as peers and routinely delaying induction of puberty is not recommended.

Many forms of oestrogen are available in both oral and transdermal forms and treatment can be initiated from 12 years of age. Typically a small dose 1/8 to 1/10 of the adult replacement dose is started and gradually titrated upwards over 2–4 years. Progesterone treatment is usually started when either breakthrough bleeding occurs or after completion of sexual maturation with 2–3 years of oestrogen treatment.

Syndromes Associated With Short Stature

Prader–Willi Syndrome

Prader–Willi syndrome (PWS) is a complex genetic disorder characterized by neonatal hypotonia, neonatal feeding difficulties with later hyperphagia, morbid obesity, short stature, hypogonadism, developmental delay, learning difficulties and psychiatric problems. Characteristic facial features are present with a thin upper lip and almond-shaped eyes. Hands and feet are small and cryptorchidism is present in 80% of males.

PWS is caused by lack of expression of paternally inherited genes on chromosome 15q11–q13, due in 75% of cases to a deletion on the paternal allele, in 24% cases to maternal uniparental disomy and in 1% to imprinting abnormalities [13] (https://doi.org/10.1210/jc.2008-0649). PWS can be caused very rarely by a paternal chromosomal translocation. The incidence is 1 in 30,000 live births.

Nasogastric tube feeding may be required in the first few months of life due to poor sucking and hypotonia. Care should be taken not to overfeed these infants due to the later hyperphagia. A programme of physiotherapy and speech and language therapy may be helpful to optimize developmental progress. Management of obesity involves introduction of a low calorie diet, regular exercise with supervision and restriction of access to food. Pharmacological treatment and bariatric surgery have not been reported as being successful. Adults with PWS have increased body fat and reduced muscle mass with a 25% incidence of type 2 diabetes by 20 years of age.

Birth size is −1.37 SDS for weight and −0.46 SDS for length. Short stature develops during childhood with 70% having biochemical evidence of GHD on pharmacological stimulation testing. Untreated final adult height is ~160 cm in males and ~150 cm in females.

GH treatment is licenced for PWS. Testing of GH status before starting therapy is not mandatory, but many centres do evaluate GH status. The aims of treatment are to improve growth and body composition. Randomized controlled trials have demonstrated efficacy in improving height and body composition. Final adult height has been reported in a small number of subjects with mean SDS −0.3 and −1.0 in two studies. Increasing evidence suggests that early introduction of GH during the first year of life can aid muscle tone and motor development.

Obstructive sleep apnoea and scoliosis are common in patients with PWS and potential complications of GH therapy. Obstructive sleep apnoea is caused by a combination of adenotonsillar hypertrophy on a background of an already narrowed upper airway, hypotonia of the airway muscles, obesity and kyphoscoliosis. There have been several reports of unexpected deaths in children with PWS, most involving respiratory infection, sleep apnoea, hypoventilation and adenotonsillar hypertrophy. Multiple studies on the effects of GH on sleep-disordered breathing have not convincingly demonstrated an overall increase in apnoea/hypopnoea associated with GH therapy but there were a small number of children where apnoea/hypopnea worsened after GH therapy and some evidence suggests a higher risk of death in the first nine months after institution of GH treatment. Close attention to symptoms of sleep-disordered breathing and polysomnography is recommended in all children with PWS, even those not starting on GH treatment. GH therapy is contraindicated in children with breathing difficulties until ENT evaluation and treatment of respiratory-compromising obesity has been achieved and should not be initiated during an acute respiratory infection but need not be routinely discontinued during subsequent episodes of respiratory infection.

GH treatment should be started at 9–12 µg/kg/day increasing to 35 µg/kg/day avoiding raised IGF-I concentrations (>+2SDS). Patients with PWS are often highly sensitive to GH therapy in terms of generation of IGF-I with many patients maintaining an IGF-I concentration in the upper half of the normal range with a modest dose of GH. Guidelines suggest aiming for an IGF-I SDS of +1 to +2 SDS in childhood and 0 to +2 SDS in adulthood [14].

Scoliosis affects up to 70% of children with PWS and arises due to the obesity and hypotonia. Treatment under the direction of a spinal surgeon may involve bracing or surgery. Development or worsening of scoliosis during GH therapy may occur but is more likely to be the natural evolution of the disorder rather than due to GH therapy and, in general, GH treatment should not be discontinued. Exclusion criteria for starting GH include severe obesity, uncontrolled diabetes, untreated severe obstructive sleep apnoea, active cancer and active psychosis. Given the multiple different clinical outcomes influenced by GH in PWS (height, body composition, psychomotor development), a definition of poor response based on growth velocity alone is not

appropriate. Consideration of all these end points should be made before discontinuing GH therapy that should typically be continued until end of growth.

GH therapy may be beneficial into adulthood with improvements in bone mass, muscle mass, body composition and cardiovascular risk. In those with childhood GHD, this persists into adulthood in >70% of patients and it may be useful to re-evaluate GH status in countries where GH therapy for an adult is available for GHD but not PWS.

Noonan Syndrome and Associated Disorders

Noonan syndrome is an autosomal dominant disorder caused by mutations in *PTPN11* (50%), *SOS1* (13%), *RAF1 (5%)*, *RIT1* (5%), *KRAS*, *NRAS* (<1%), *BRAF* (<1%) and *MAP2K1* (<1%) with the remaining cases as yet unexplained. Clinical features are listed in Table 6.2 and the RAS pathway is summarized in Figure 6.5.

The incidence is estimated to be 1:1000 to 1:2500. Birth weight and length are usually normal but feeding difficulties are common in infancy with postnatal growth failure evolving after the first year of life. Mean height follows the third centile until puberty when there is a delayed and reduced pubertal growth spurt. Bone age is typically significantly delayed. Final adult height without GH treatment is 161–167 cm in males and 150–155 cm in females and Noonan syndrome-specific growth charts are available. Risk of childhood malignancy is approximately eightfold higher in Noonan syndrome with an increase in leukaemias, rhabdomyosarcoma and neuroblastoma.

Short stature due to Noonan syndrome is a licenced indication for GH therapy in the USA and Japan, and elsewhere off-licence treatment may be considered. Short-term improvement in height velocity has been demonstrated with an increase in height SDS over 1 year of treatment of 0.35 SD. Studies following patients to adult height suggest a gain of 0.6–1.7 SD while the increase in height SDS in data from the Kabi International Growth Study was 0.6 SD by normal population standards and 1.0 SD using Noonan syndrome-specific standards. There has not been evidence of increased malignancy risk associated with GH therapy in Noonan syndrome; however, caution is advised as the number of patients treated remains smaller than for other GH indications. The issues surrounding GH and malignancy risk should be discussed with patients and their families before starting therapy. Additionally, children with a cardiomyopathy should not be commenced on GH treatment.

There is clinical overlap with other disorders of the RAS-RAF-MAPK cascade (e.g. LEOPARD syndrome, cardiofaciocutaneous (CFC) syndrome and Costello syndrome). Patients with CFC syndrome share similar cardiac and facial features but have a higher risk of intellectual disability and central nervous system abnormalities, while skin and gastrointestinal abnormalities are more florid. Skin is keratotic and ichthyotic with sparse, curly brittle hair. Gastrointestinal complications include herniae, failure to thrive, oral aversion, malrotation, constipation and reflux. LEOPARD syndrome (*l*entigines, *E*CG abnormalities, *o*cular hypertelorism, *p*ulmonary stenosis, *a*bnormalities of genitalia, *r*etardation of growth, *d*eafness) is also known as Noonan syndrome with multiple lentigines. In childhood, LEOPARD and Noonan syndrome features overlap but with age, other characteristic features including lentigines and hearing loss develop. Costello syndrome is characterized by developmental delay and intellectual disability, loose folds of skin (particularly on the hands and feet), hypermobility, facial dysmorphism, cardiomyopathy and facial warts. Costello syndrome is also associated with increased risk of malignancy.

Table 6.2 Clinical features of Noonan syndrome.

Short stature
Broad webbed neck
Congenital heart disease (most commonly pulmonary valve stenosis, atrial septal defects and hypertrophic cardiomyopathy)
Developmental delay
Pectus abnormalities
Widely set nipples
Cryptorchidism
Characteristic facial features including low-set, posteriorly rotated ears with fleshy helices, vivid blue or blue-green irises and eyes that are often wide-spaced, down-slanted, and with epicanthic folds and ptosis
Thin scalp hair during infancy that becomes thicker and curly during childhood/adolescence

Primordial Growth Disorders

A number of conditions are associated with intra- and extrauterine growth failure with characteristic features and other system disorders. There is evidence for efficacy of GH in SRS but limited data in other conditions and GH is contraindicated in conditions carrying a risk of malignancy.

Silver–Russell Syndrome

SRS (OMIM 180860) is a disorder of intra- and extrauterine growth impairment with the child born SGA. There is a distinctive facial appearance with a triangular-shaped face, broad forehead, small pointed chin and wide thin mouth. Intelligence is normal or mildly impaired. Feeding difficulties and body asymmetry are also common. Severity varies and the phenotype may be less distinct in adolescents and adults.

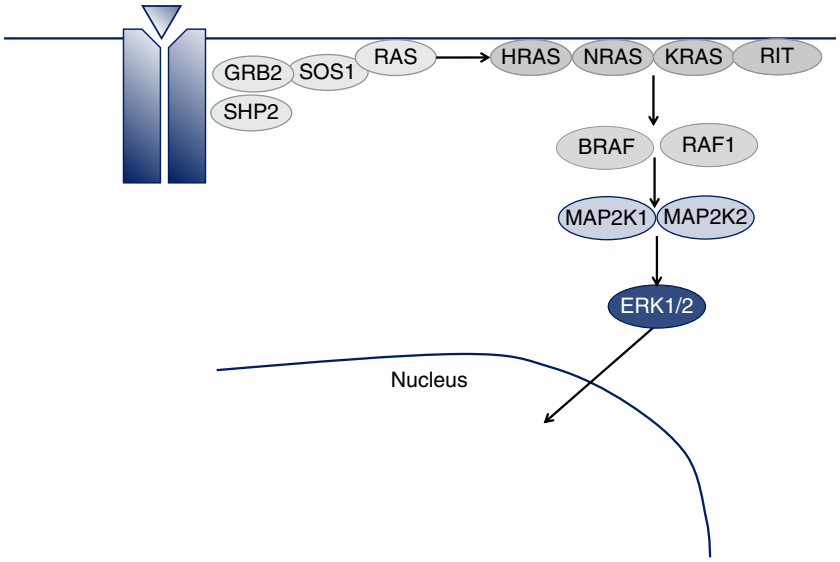

Figure 6.5 The RAS/MAPK pathway. The pathway is activated by binding of a ligand to transmembrane receptor (e.g. GHR). Phosphorylation of the intacellular domain of the receptor leads to recruitment and binding of adaptor proteins including SHP2 (encoded by *PTPN11*), GRB2 and SOS1. A RAS family protein (HRAS, NRAS, KRAS or RIT1) translocates to the nucleus. The activated adaptor protein complex transfers a GTP molecule to the inactive GDP bound RAS protein. The activated GTP bound RAS protein activates the MAPK cascade which includes BRAF, RAF1 and MAP2K1 (all involved in Noonan syndrome pathogenesis).

SRS is caused by hypomethylation of the paternally methylated H19/IGF2 intergenic differentially methylated region at chromosome 11p15 in 50% of cases. Absence of methylation at this region leads to binding of CCCTC-binding factor that blocks access to *IGF2* promoters. *IGF2* is normally expressed from the paternal allele that is methylated but hypomethylation in SRS leads to suppression of *IGF2* transcription and an increase in transcription of the noncoding RNA H19.

The second most frequent molecular cause of SRS is maternal uniparental disomy of chromosome 7 (5–10% of cases). Rare molecular causes of SRS include maternally inherited mutations of *CDKN1C* (a maternally expressed growth factor located on the centromeric domain of 11p15) and paternally inherited mutations of *IGF2*. Only one family each with *CDKN1C* and *IGF2* mutations has been reported.

Several scoring systems have been developed to aid diagnosis and consensus guidelines recommend use of the Netchine–Harbison scoring system. One point is scored for meeting each of the following:

1) Small size at birth – birth weight/length ≦ –2 SDS.
2) Postnatal growth impairment as evidenced by a height at 24 months ≦–2 SD or height SDS more than 2 SD below mid-parental height SDS.
3) Relative macrocephaly at birth with OFC ≧ 1.5 SDS above birth weight/length SDS.
4) Protruding forehead – forehead projecting beyond facial plane on side view as a toddler.
5) Body asymmetry – leg length discrepancy (LLD) of ≥0.5 cm *or* arm asymmetry *or* LLD < 0.5 cm with at least two other asymmetrical body parts (one non-face).
6) Feeding difficulties and/or low BMI – BM ≤ –2SDS at 24 months OR current use of a feeding tube or medication for appetite stimulation.

For a score of 4/6 or 3/6 (with strong clinical suspicion), molecular testing is appropriate, examining for maternal uniparental disomy of chromosome 7 and 11p15 hypomethylation. *CDKN1C* and *IGF2* sequencing should be considered where multiple affected members are reported within a family. For those who test negative for 11p15 hypomethylation or maternal uniparental disomy of chromosome 7, a clinical diagnosis can be made where the score is 5/6 or 4/6 and where the patient has both relative macrocephaly and a protruding forehead. A diagnostic flow chart was produced as part of the recent consensus guidelines [15] (http://www.nature.com/nrendo/journal/v13/n2/full/nrendo.2016.138.html) (see Figure 6.6).

For the first 2 years of life, the goals of treatment are to support nutrition to prevent hypoglycaemia and optimize growth before starting GH. Children with SRS are normally slender and care needs to be taken not to overfeed patients. The aim should be a weight 75–85% of the 50th centile weight for length/height and/or BMI 12–14 kg/m^2. Initial treatment is with nutritional supplements but SRS patients have poor appetites and gastrostomy feeds may be required. Appetite stimulants are not recommended due to a lack of published data. Hypoglycaemia can be prevented by frequent feeds or the addition of high molecular weight glucose polymers to the late-night feed. Children with SRS often require admission before surgery so that 10% intravenous dextrose can be administered during the fasting period.

SRS children were included in the trials of GH that led to the licensing of GH for SGA children. Patients with SRS should be offered GH under the SGA licence. The aim of the age cut-off to start GH under this licence was to avoid the administration of GH to individuals who may experience catch-up growth. Since catch-up growth

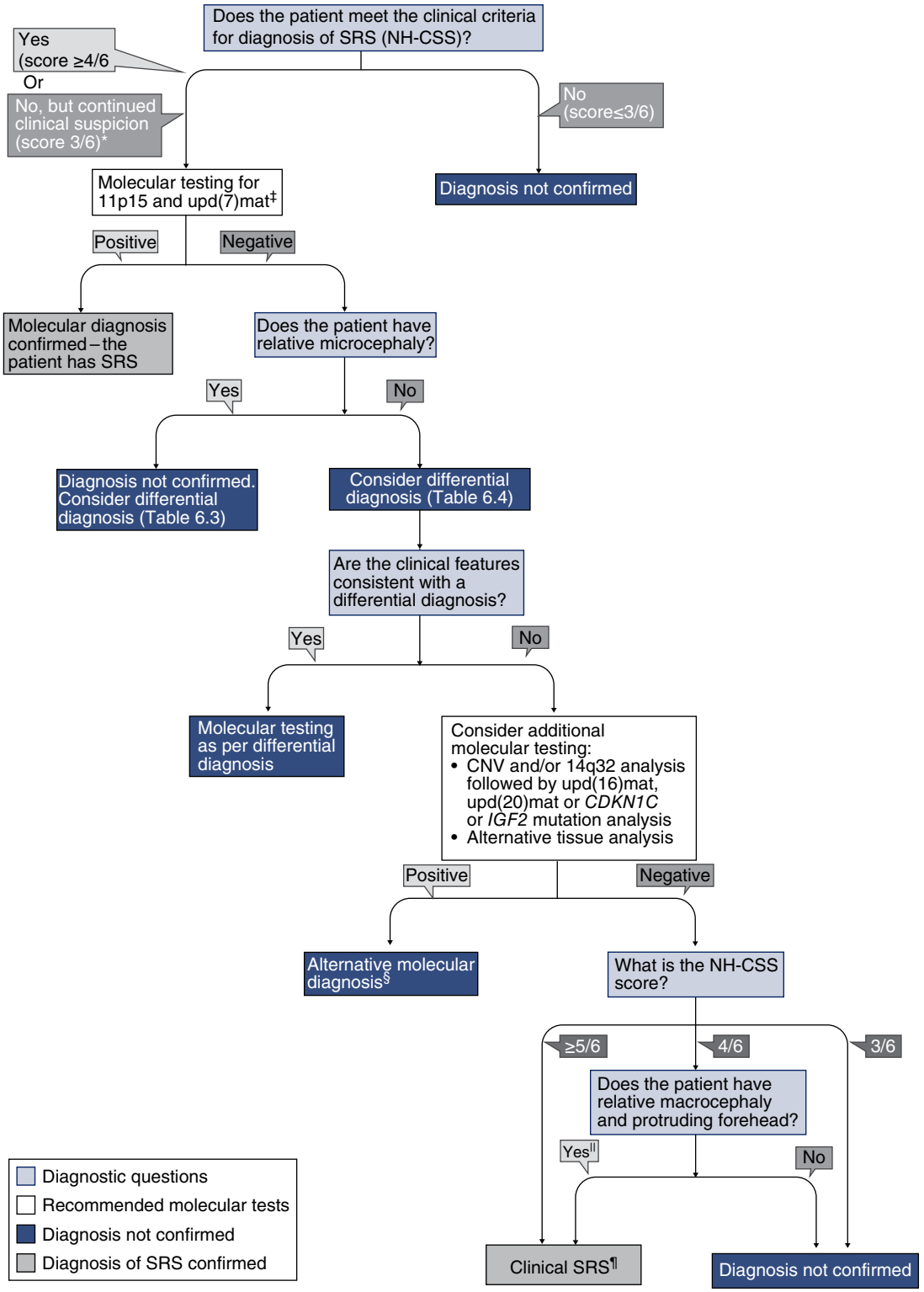

Figure 6.6 Flow chart for investigation and diagnosis of SRS. *Studies have excluded 11p15 LOM and upd(7)mat in patients with intrauterine growth retardation and postnatal growth retardation alone; some patients, particularly those with upd(7)mat or children under 2 years, score 3/6 (see text for details). ‡Arrange CNV analysis before other investigations if patient has notable unexplained global developmental delay and/or intellectual disability and/or relative microcephaly. §Insufficient evidence at present to determine relationship to SRS, with the exception of tissue mosaicism for 11p15 LOM. ‖Unless evidence of catch-up growth by 2 years. ¶Previously known as idiopathic SRS. CNV, copy number variant; LOM, loss of methylation; NH-CSS, Netchine–Harbison clinical scoring system; SRS, Silver–Russell syndrome. *Source:* Reproduced with permission of Wakeling et al. [15].

is extremely unlikely in patients with SRS, early administration of GH should be considered, especially in children with hypoglycaemia or severe growth restriction. GH improves height, body composition, appetite and muscle power. Patients with SRS experience early adrenarche with bone age advancement and an earlier onset into puberty with rapid progression that has led to a consideration of the use of GnRH agonist therapy to delay it, but there is a lack of evidence of efficacy.

3-M Syndrome

3-M syndrome is an autosomal recessive disorder causing pre- and postnatal growth restriction with dysmorphic facial features (broad forehead, fleshy upturned nose, full fleshy lips, triangular face, midface hypoplasia, long philtrum) and normal intelligence. Prominent heels are found in all patients but can also be seen in patients with SRS due to maternal uniparental disomy of chromosome 7. Radiological findings may include slender long bones and relatively tall vertebral bodies.

3-M syndrome is caused by loss-of-function mutations in *CUL7*, *OBSL1* and *CCDC8* but the mechanism by which they lead to growth failure is unclear. Growth impairment is most severe in patients with *CUL7* mutations. Some response to GH therapy is seen in a small proportion of patients and a trial of GH may be appropriate.

Mulibrey Nanism

Mulibrey (muscle–liver–brain–eye) nanism (OMIM 253520) is an autosomal recessive disorder caused by mutations in tripartite motif 37 (*TRIM37*). Affected individuals have severe intra- and extrauterine growth failure with normal intelligence, a characteristic facial appearance (triangular face, high broad forehead and low nasal bridge), high pitched voice, hypotonia, hepatomegaly, congestive heart failure due to constrictive pericarditis and cutaneous naevi flammei. Radiological findings may include slender long bones with a relatively thick cortex and fibrous dysplasia.

The incidence of Mulibrey nanism is 1:40,000 in the Finnish population but very few cases have been reported outside Finland. Diagnostic criteria have been defined and three major signs with one minor sign or two major signs with three minor signs are required for diagnosis:

Major signs

- Growth failure (A or B or C): (A) SGA lacking catch-up growth, (B) height in childhood <−2.5 SDS or (C) height in adulthood <−3.0 SDS.
- Characteristic radiological findings (A or B): (A) slender long bones with thick cortex and narrow medullar channels or (B) low and shallow (J-shaped) sella turcica.
- Characteristic craniofacial features: scaphocephaly, triangular face, high and broad forehead, low nasal bridge and telecanthus.
- Characteristic ocular findings: yellowish dots in the mid-peripheral region of the retina.
- Affected sibling.

Minor signs

- Peculiar high pitched voice.
- Hepatomegaly.
- Cutaneous naevi flammei.
- Fibrous dysplasia of the long bones.

Feeding difficulties affect a third of babies in the newborn period and, by the age of 2, over half of affected children fail to thrive. Upper respiratory tract infections are common with half of the children diagnosed with pneumonia and one quarter suffering at least one episode of respiratory failure induced by infection by two years of age. Psychomotor development is normal or mildly impaired. Mean birth length SDS is −3.1 and progressive growth failure leads to a mean height SDS at two years of −4.4. Treatment with GH results in a modest increase in final height of +0.6 SD.

SHORT Syndrome

Short stature, hyperextensibility, hernia, ocular depression, Rieger anomaly and teething delay (SHORT) syndrome is an autosomal dominant disorder caused by mutations in *PIK3R1*, a regulatory subunit of phosphatidylinositol 3 kinase, a component of the IGF-I and insulin signal transduction systems. Facial features include micrognathia, high broad forehead, triangular-shaped face, deep-set eyes, prominent nose, low-set posteriorly rotated ears, hypoplastic nasal alae, facial lipodystrophy and thin hair. Other clinical features not included in the acronym are partial lipodystrophy, transparent skin, dimples on the elbows and buttocks, fifth finger clinodactyly, hypogonadism, high pitched voice, type 2 diabetes, nephrocalcinosis and thin gracile bones. Birth weight is low with a mean of −3.3 SD and postnatal growth is impaired with an average final height of 154 cm. Intelligence is normal.

Floating–Harbor Syndrome

Floating–Harbor syndrome is a disorder of pre- and postnatal growth impairment with speech delay, delayed bone age and a characteristic facial appearance with triangular face, deep-set eyes, long eyelashes, bulbous nose, wide columella, short philtrum and thin lips. It is

autosomal dominant and caused by mutations in *SRCAP*, which encodes Snf2 CREBBP-activating protein that is involved in DNA repair and activation of CREB-binding protein. Mutations in *CREBBP* cause Rubinstein–Taybi syndrome.

IMAGe Syndrome

Patients present with a combination of intrauterine growth impairment, metaphyseal dysplasia, congenital adrenal hypoplasia and genital anomalies. Pre- and postnatal growth impairment is seen in all patients and intelligence is normal or mildly impaired. The disorder is caused by maternally imprinted mutations in *CDKN1C*. Facial features include frontal bossing, low-set ears, flat nasal bridge and a short nose.

Bloom Syndrome

Bloom syndrome (OMIM 210900) is an autosomal recessive condition characterized by pre- and postnatal growth restriction and a thin face with malar hypoplasia. Other features include sun-sensitive telangiectasia, hypo- and hyperpigmented skin, predisposition to malignancy (generalized increased incidence for all types of malignant disease) and chromosomal instability. Incidence is 1:160,000 but the condition is rarely found outside the Ashkenazi Jewish population. Most affected individuals die in the third decade of life from malignancy.

The diagnosis is made by a combination of clinical features (no formal diagnostic criteria) and demonstration of chromosomal instability or direct gene sequencing. The mutation affects the *BLM* gene that encodes a member of the RecQ DNA helicase enzymes that are responsible for separating complementary strands of DNA in processes such as DNA replication, transcription, genetic recombination and DNA repair. GH is contraindicated.

Microcephalic Osteodysplastic Primordial Dwarfism Type II

Microcephalic osteodysplastic primordial dwarfism type II (MOPD II) is another autosomal recessive condition of severe intra- and extrauterine growth impairment with affected individuals being ~100 cm tall in adulthood. Other characteristic features include normal intelligence, a high pitched voice, abnormal dentition, disproportionate shortening of the forearms and legs, prominent eyes and a prominent nose.

MOPD II is caused by nonsense mutations in the pericentrin gene. Pericentrin is a giant 370 kDA coiled protein that localizes to the centrosomes. Lack of it results in abnormalities of the mitotic spindle and it is likely that this is a disorder of impaired cell proliferation. Individuals are at increased risk of intracranial aneurysms and type II diabetes; screening for both conditions is advised. Treatment with GH is usually ineffective.

Seckel Syndrome

Seckel syndrome is phenotypically very similar to MOPD type II with severe pre and postnatal growth restriction, prominent nose, prominent eyes, micrognathia and microcephaly. In contrast to MOPD type II, affected individuals have moderate to severe learning difficulties. Seckel syndrome is caused by a splice mutation in ataxia telangiectasia and Rad-3-related protein and nonsense mutations in pericentrin.

Meier–Gorlin Syndrome

Meier–Gorlin syndrome is an autosomal recessive disorder of pre- and postnatal growth with microcephaly, microtia, a narrow beaked nose with low insertion of the columella, small mouth and retrognathia. Other clinical features include patellar hypoplasia, cryptorchidism and mammary hypoplasia. It is caused by mutations in *ORC1*, *ORC4*, *ORC6*, *CDT1* and *CDC6*. These genes all encode components of the origin recognition complex, a multisubunit DNA-binding complex that is an essential component for DNA replication.

Nijmegen Breakage Syndrome

Nijmegen breakage syndrome is a chromosomal stability disorder with immunodeficiency and increased cancer risk. Affected individuals are born with a low birth weight (mean –1.6 SDS). Severe progressive microcephaly is associated with the short stature. Intelligence may be normal during infancy but there is a progressive deterioration. Facial features include a receding forehead, prominent midface, small mandible, up-slanting palpebral fissures, long nose and philtrum and large ears. The disorder is autosomal recessive and caused by mutations in *NBN*.

Fanconi Anaemia

Fanconi anaemia (FA) can present with short stature of pre- or postnatal onset. Short stature is present in ~75% of individuals. Other features include abnormal skin pigmentation (café au lait macules or areas of hypopigmentation), radial ray abnormalities (hypoplastic thumb or radius), microcephaly, ophthalmic abnormalities (microphthalmia, cataracts), genitourinary abnormalities (horseshoe kidney, cryptorchidism,

hypospadias), progressive bone marrow failure, aplastic anaemia, leukaemia and early onset solid tumours. Inheritance can be autosomal recessive (16 genes), autosomal dominant (1 gene) or X-linked (1 gene). The diagnosis is established via chromosomal breakage studies with mitomycin C or diepoxybutane or via genetic sequencing.

Idiopathic Short Stature

ISS can be defined as a height more than two standard deviations below the mean for age and gender where no aetiology has been identified and can include patients with familial short stature, constitutional delay in growth and adolescence and patients who are born SGA. In Europe GH is licensed for treatment of the SGA child but not for ISS, while GH therapy is licensed for both groups of patients in the USA.

The typical growth of a child with ISS is that growth falters in childhood and height falls to below −2 SD and either remains there growing parallel to but below the lowest centile of a normal growth chart or may continue slowly to decline. Some children with ISS catch up during puberty. At the end of growth, ~66% of children with ISS reach a height within the normal adult range around −1.5 SD.

There are abnormalities in both the GH-IGF-I axis and within the growth plate in children with ISS. GH secretion and GH responsiveness are continuous. Children with ISS are not GH deficient and do not have Laron syndrome or other causes of primary IGF-I deficiency, but a combination of minor defects in GH secretion/responsiveness and IGF-I secretion/responsiveness may contribute to the growth impairment.

IGF-I concentrations are low in 50% of children with ISS, while spontaneous GH secretion is normal or increased. Acute responses to GH administration in the context of an IGF generation test may show low rises in serum IGF-I and IGFBP-3. Genetic defects within the GH-IGF pathway have been identified in some patients previously classed as ISS with heterozygous mutations in *IGF1* and *IGF1R* reported. Defining the GH-IGF-I pathway status has been suggested as a mode to select treatment with GH or IGF-I (or a combination of both) but there is increasing evidence that the primary pathology for ISS may lie within the growth plate rather than the GH-IGF1 axis.

The physical examination should seek subtle dysmorphism, disproportion and the phenotypic features of GHD. In all patients, laboratory evaluation including full blood count, kidney, liver and bone, TSH, free T_4 and IGF-I concentrations should be taken. Laboratory evaluation for coeliac disease and a bone age should also be measured. A karyotype should be examined in all female patients to exclude Turner syndrome. GHD must be excluded. For some children a diagnosis of GHD can be excluded on the basis of an IGF-I concentration and auxology but others will require formal GH stimulation testing.

Monogenic disorders currently identified within cohorts of children labelled as ISS include *SHOX/PAR1* abnormalities, *NPR2* mutations and *ACAN* mutations. The first to be identified were *SHOX* mutations that are thought to account for 3–15% of patients. In addition to mutations and deletions affecting the *SHOX* coding region, deletions and duplications affecting the upstream and downstream conserved noncoding regulatory elements of the pseudoautosomal region 1 can also cause *SHOX* deficiency. Mutations in *SHOX* were identified simultaneously in both ISS patients and children with Leri–Weill dyschondrosteosis (LWD).

Mutations in the second gene identified in ISS patients, *NPR2*, were identified first in patients with acromesomelic dysplasia. *NPR2* encodes the receptor for C-type natriuretic peptide and signalling through this receptor antagonizes signalling via the fibroblast growth factor receptor 3. Activating mutations in *FGFR3* cause growth impairment by increased signal transduction and it is likely that loss-of-function mutations in *NPR2* lead to growth impairment by loss of this antagonism and uncontrolled *FGFR3* signal transduction. Six percent of patients with ISS are thought to harbour *NPR2* mutations.

The frequency of mutations for both *SHOX* and *NPR2* depends on the phenotype of the patients evaluated; prevalence of mutations is increased in the presence of disproportionate short stature or skeletal abnormalities.

ACAN encodes aggrecan, a proteoglycan component of the extracellular matrix. While *ACAN* mutations have been associated with skeletal dysplasias, including spondyloepimetaphyseal dysplasia and spondyloepiphyseal dysplasia, they can also present with short stature with an advanced bone age but without other specific features on skeletal survey. Birth size is in the lower half of the normal range and patients present with short stature without disproportion in childhood. Bone age is advanced in all patients but in 1/3 of patients the bone age is <1 year advanced.

High-throughput sequencing techniques of either whole exome or targeted panels of short stature-related genes have been used to identify other genetic causes of patients labelled as ISS. The yield from targeted gene panels has been low at ~2%, with Noonan syndrome the commonest identified genetic cause. Whole exome sequencing has a greater reported yield at 36% with

3-M, Kenny–Caffey and Floating–Harbor syndromes identified in ISS patients. As knowledge of the genetic aetiology of short stature increases, it is likely that the yield from these investigations will improve.

Although whole exome sequencing is not yet routine, analysis of CNVs is widely available. CNVs are areas of genomic DNA with an increase or decrease in copy number varying in size from kilobase to megabase. CNVs can be subdivided into common CNVs with a population frequency of >1% and copy number states ranging from 0 to 30 copies per diploid genome and rare CNVs (<1%) with a lower range of copy number states (usually 0–3 copies per genome). CNVs can cause disease by altering copy number of genes that are dosage sensitive, altering the expression concentration of a nearby gene through position effect and unmasking the effects of recessive mutation on the remaining undeleted allele or by a transversion effect (deleting elements required for communication between alleles on homologous chromosomes).

65–80% of the normal population carries a rare CNV <100 kb with 5–10% carrying a CNV <500 kb and ~1% of individuals carrying a CNV of ≥1Mb. Differentiating benign and pathogenic CNVs can be challenging due to variable penetrance of the phenotype, a benign CNV being inherited in the homozygous state or present on the X chromosome in a boy when inherited from his mother or inherited in combination with another CNV.

Two main technologies exist to assess CNVs – array comparative genomic hybridization and SNP arrays. In array CGH, patient and control DNA is labelled with differently coloured fluorescent dyes and hybridized to a microarray slide containing DNA probes covering the entire genome. CNVs are detected as differences in the ratio of fluorescent dyes for each probe. High-density SNP arrays use only patient DNA that is fluorescently labelled and hybridized to a chip with probes covering SNPs spread throughout the genome. Areas of copy number gain or loss are identified by areas of contiguous SNPs where the fluorescence intensity is altered in comparison with the normalized intensity for the whole genome. In addition to detecting CNVs, unlike array CGH, SNP arrays can also be used to identify copy number neutral chromosomal aberrations such as uniparental disomy or loss of heterozygosity.

Several studies have now identified pathological CNVs in 10–15% of patients with ISS. *SHOX* and *IGF1R* deletions are the most common genes identified with other causes including SRS (maternal UPD chromosome 7), Temple syndrome (maternal uniparental disomy chromosome 14) and 22q11 deletion. It would be reasonable to add CNV analysis to the routine investigations undertaken in ISS patients.

Treatment of ISS

Whether or not to treat ISS children with GH remains controversial with GH treatment licensed in some countries but not others. Expert opinion [16] varied between −2 and −3 SD as the threshold for considering treatment. The US FDA licence currently uses a height <−2.25 SD as the threshold for GH therapy. Where there is an element of constitutional delay and the predicted adult height is within the normal range, a watch-and-wait approach should be considered. Where findings point to a potential diagnosis of skeletal dysplasia or a condition associated with cancer risk, children should not be treated with GH therapy due to the lack of efficacy in the former group and increased risk in the latter group. Treatment is most important for children with the greatest impairment of height where a very low adult height may impede everyday activities. As treatment response is greater at younger ages and this allows longer duration of therapy, treatment should be initiated as early as possible in children with severe growth impairment who are likely to respond to rhGH treatment.

The response to GH therapy is variable; there is uncertainty as to the exact long-term benefit of the intervention and long-term safety is not assured. Many of these issues stem from the fact that ISS represents a mixture of pathologies that affect growth. Many short-term studies report an increase in height velocity associated with GH treatment and a small number of long-term studies in a limited number of individuals indicate an increase in height SDS over ~5 years of treatment of +0.5 to +1.2 SD using a dose range of 30–67 μg/kg/day.

Recombinant human IGF-I has been used in one trial of ISS patients with low serum IGF-I concentrations. A dose of 120 μg/kg twice daily increased height velocity to 7.9 cm/year, higher than untreated controls at 5.2 cm/year; this was a therapeutic response similar to that seen in treatment of primary IGF-I deficiency. Longer-term trials of IGF-I are lacking and there are no trials directly comparing IGF-I with GH therapy.

A combination of GH and IGF-I treatment in ISS patients with an IGF-I SDS < −1 reported a height gain of +1.9 SD over 3 years in the combination therapy arm (45 μg/kg/day GH and 150 μg/kg/day IGF-I) compared with +1.3 SD in the GH therapy arm. Although the data on IGF-I are promising, GH should remain the first choice of treatment because GH is probably more efficacious than IGF-I monotherapy, and the experience with using GH therapy is vastly greater than IGF-I.

Other therapeutic interventions have included GnRH analogues and aromatase inhibitors. GnRH analogue therapy has been proposed either alone or in combination with GH therapy. The effects on height gain are, at best, modest and to obtain clinically significant

improvements in height requires several years of treatment. The improvement in height must be balanced with the distress caused to the young person by delaying puberty. Long-term effects on bone health also need consideration.

Aromatase inhibitors have the advantage of not delaying puberty in boys, but carry a risk of ovarian cyst development in girls. They appear to be more effective at promoting height gain but at present these data are mostly limited to short-term outcomes with final height reported in only one study. Mild abnormalities of vertebrae were found in one study in 45% of patients treated with letrozole compared with 0% in the control group. Currently neither GnRH analogue therapy nor aromatase inhibitors can be recommended in the treatment of ISS.

Skeletal Dysplasias

Skeletal dysplasias are a heterogeneous group of over 450 genetic disorders characterized by abnormal growth, development, differentiation and maintenance of the bone and cartilage. While they predominantly affect the bone, skeletal dysplasias can also have an effect on the muscles, ligaments and tendons. The incidence is around 1:5000 live births. Severity and presentation vary, with the most severe cases detected *in utero* with short limbs on ultrasound. Most patients present with short stature in childhood, but some present only with premature arthritis in adult life.

The spine and limbs are frequently affected and, while patients with endocrine disease typically present with proportionate short stature, those with skeletal dysplasias present with disproportionate short stature that can be divided into those with short limbs or those with a short trunk. Routine auxology should include measurement of sitting height, upper/lower segment ratio and arm span. The lower segment is measured from symphysis pubis to heel with the upper segment calculated as total height – lower segment.

Short-limbed disproportionate short stature can be divided into rhizomelia (short proximal segments – humerus/femur), mesomelia (middle segment shortening – ulna/radius/tibia/fibula) and acromelia (shortening of distal segment – hands/feet). Brachydactyly refers to short digits and micromelia to short limbs.

Evaluation of the child suspected of having a skeletal dysplasia includes careful clinical examination for associated features such as macrocephaly, cleft palate, micrognathia, flat nasal bridge, midface hypoplasia and dentinogenesis imperfecta. Abnormalities of the hair, nails and skin can also be seen. Congenital heart disease, genital abnormalities, Hirschsprung disease and immune deficiency can all be associated with individual skeletal dysplasias and can guide diagnosis. A radiographic skeletal survey should be performed.

Terms used include spondylo, referring to the spine; epiphyseal, referring to absent, small or irregularly ossified epiphyses; metaphyseal, referring to irregular, widened or flared metaphyses; and diaphyseal, referring to widening, sclerosis, cortical thickening or medullary narrowing or expansion of the diaphyses.

Skeletal Dysplasia Classification

The first eight groups of conditions in the 2010 nosology are separated according to the molecular basis of the disease: *FGFR3*, type 2 collagen, type 11 collagen, sulphation disorders, perlecan, aggrecan, filamin and *TRPV4*. The other 32 groups are organized according to their clinical and radiographic presentation. The prefix *acro-* refers to the extremities (hands and feet), *meso-* to the middle portion (ulna and radius, tibia and fibula), *rhizo-* to the proximal portion (femur and humerus), *spondylo-* to the spine, *epi-* to the epiphyses and *meta-* to the metaphyses. For example, if only the hands and feet are shorter, one would consult the acromelic group of conditions, whereas if the spine and metaphyses are affected, one would consult the spondylometaphyseal dysplasias. Listed below are the 40 groups of conditions to be detailed in this chapter.

Over 350 genes responsible for skeletal dysplasias have been identified. With the overlapping phenotype of many genetic causes of skeletal dysplasia, these disorders will particularly benefit from increasing availability of targeted gene panels with exome sequencing.

The classification of skeletal dysplasias includes groups defined by their molecular basis and those defined by their clinical and radiographic presentation (Tables 6.3 and 6.4).

A selection of the more common skeletal disorders is described below.

Table 6.3 Groups of conditions organized according to their molecular basis.

FGFR3 chondrodysplasia group
Type 2 collagen group and similar disorders
Type 11 collagen group
Sulphation disorders group
Perlecan group
Aggrecan group
Filamin group and related disorders
TRPV4 group

Table 6.4 Groups of conditions organized according to their clinical presentations.

Short-rib dysplasias (with or without polydactyly) group
Multiple epiphyseal dysplasia and pseudoachondroplasia group
Metaphyseal dysplasias
Spondylometaphyseal dysplasias
Spondylo-epi-(meta)-physeal dysplasias
Severe spondylodysplastic dysplasias
Acromelic dysplasias (extremities of the limbs)
Acromesomelic dysplasias (extremities and middle portion of the limbs)
Mesomelic and rhizo-mesomelic dysplasias (proximal and middle portions of the limbs)
Bent bones dysplasias
Slender bone dysplasia group
Dysplasias with multiple joint dislocations
Chondrodysplasia punctata (CDP) group
Neonatal osteosclerotic dysplasias
Increased bone density group (without modification of bone shape)
Increased bone density group with metaphyseal and/or diaphyseal involvement
Osteogenesis imperfecta and decreased bone density group
Abnormal mineralization group
Lysosomal storage diseases with skeletal involvement (dysostosis multiplex group)
Osteolysis group
Disorganized development of skeletal components group
Overgrowth syndromes with skeletal involvement
Genetic inflammatory/rheumatoid-like osteoarthropathies
Cleidocranial dysplasia and isolated cranial ossification defects group
Craniosynostosis syndromes
Dysostoses with predominant craniofacial involvement
Dysostoses with predominant vertebral with and without costal involvement
Patellar dysostoses
Brachydactylies (with or without extraskeletal manifestations)
Limb hypoplasia – reduction defects group
Polydactyly-syndactyly-triphalangism group
Defects in joint formation and synostoses

Achondroplasia

Achondroplasia is an autosomal dominant disorder caused by activating mutations in fibroblast growth factor receptor 3 (*FGFR3*). It is the commonest cause of disproportionate short stature. Affected individuals have rhizomelic shortening of limbs, macrocephaly and characteristic facial features with frontal bossing and midface hypoplasia. Other clinical features include limitation of elbow extension, short fingers, trident configuration of hands, genu varum and exaggerated lumbar lordosis. Radiographic findings include short tubular bones, narrowing of the interpedicular distance of the caudal spine, rounded ilia and horizontal acetabula, narrow sacrosciatic notch, proximal femoral radiolucency and mild, generalized metaphyseal changes. Initial genetic testing is for two common mutations c.1138G > A and c.1138G > C with sequencing of the full gene only where clinical suspicion is high and initial testing for common mutations negative.

Average adult height for men with achondroplasia is 131 ± 5.6 and 124 ± 5.9 cm for women. GH therapy produces an initial improvement of height velocity but with limited long-term impact on final height – 5 years of treatment improving height by 1 SD. Limb-lengthening surgery has the potential to increase height but risks serious complications including limb loss.

One novel therapy currently in clinical trials is the C-type natriuretic peptide analogue BMN111 (vosoritide). Constitutive activation of pathways downstream of FGFR3 causes achondroplasia. C-type natriuretic peptide acts via the NPR2 receptor to downregulate MAPK signal transduction, one of the pathways activated by FGFR3. In phase 2 studies, BMN111 has been shown to increase growth velocity in achondroplasia.

Hypochondroplasia

Hypochondroplasia is also an autosomal dominant skeletal dysplasia characterized by short stature, stocky build, rhizomelic and mesomelic limb shortening, brachydactyly and macrocephaly. Radiological features include shortening of long bones with mild metaphyseal flare (especially femora and tibiae); narrowing or failure to widen the inferior lumbar interpedicular distances; mild to moderate brachydactyly; short, broad femoral neck and squared, shortened ilia. Seventy percent of cases are associated with activating *FGFR3* mutations.

Birth weight and length are often within the normal range and the disproportion in limb-to-trunk length is often mild and easily overlooked during infancy, but short stature evolves during childhood. The adult height for men with hypochondroplasia ranges from 138 to 165 cm and for adult women from 128 to 151 cm. GH therapy at 50 µg/kg/day has been reported to increase height by 0.6 SD over 3 years. Data are less extensive than those available in achondroplasia but meta-analysis suggests a modest impact on height.

Leri–Weill Dyschondrosteosis and *SHOX* Deficiency

The spectrum of short stature disorders caused by *SHOX* deficiency ranges from ISS to LWD and Langer mesomelic dysplasia. LWD is an autosomal dominant disorder caused by loss-of-function mutations or deletions affecting *SHOX* and should be considered in any child with a combination of short stature, mesomelic limb shortening and Madelung deformity or other radiographic features consistent with LWD. Birth size is within normal limits but progressive short stature develops during childhood with a blunted pubertal growth spurt and a final adult height of −2.8 SD in females and −2.3 SD in males. The Madelung deformity evolves during childhood with initial limitation to pronation/supination, eventually developing in puberty to the classic 'dinner fork' sign due to subluxation of the ulna. Radiographic criteria for Madelung deformity include:

- Wedged carpal bones.
- Triangularization of radial/ulnar distal epiphysis.
- Subluxed radial/ulnar articulation.
- Decreased length ulna/radius.
- Fusion ulnar half of radial distal epiphysis.

Other clinical features of LWD include a high-arched palate, micrognathia, short fourth metacarpals, lower leg bowing and cubitus valgus. A scoring system has been developed to define the likelihood of an *SHOX* mutation in those children with short stature without a clear diagnosis of LWD (Table 6.5).

SHOX is located on the pseudoautosomal region of X and Y chromosomes. Thus women have two copies of *SHOX*, one on each X chromosome, while men also have two copies of *SHOX*, one on the X chromosome and one on the Y chromosome. Eighty percent of individuals with short stature due to *SHOX* deficiency will have a deletion affecting *SHOX*. Many of these deletions are large enough to be detected by array CGH or SNP arrays but some smaller deletions may be detected only with multiplex ligation-dependent probe amplification. Duplication and deletions of upstream and downstream regulatory elements of *SHOX* have also been identified in individuals with LWD and ISS. For those without any CNV, sequence analysis of *SHOX* should be undertaken.

GH is licenced for treatment of short stature due to *SHOX* deficiency with similar efficacy to that seen in children treated for Turner syndrome. Increase in final height is in the order of 7–10 cm. The recommended dose is 50 µg/kg/day.

Biallelic mutations or deletions affecting *SHOX* cause Langer mesomelic dysplasia. This is a more severe skeletal dysplasia with a final height SDS from −5 to −9 SD. Severe shortening of the forearms and lower legs is seen with aplasia or severe hypoplasia of ulna and fibula. Madelung deformity is less common. The radius and ulna are typically short, thickened and curved.

Table 6.5 Scoring system to aid in selection for genetic analysis of *SHOX* in patients with short stature.

Score item	Criterion	Points
Arm span/height ratio	<96.5%	2
Sitting height/height ratio	>55.5%	2
Body mass index	>50th Centile	4
Cubitus valgus	Yes	2
Short forearm	Yes	3
Bowing of forearm	Yes	3
Appearance of muscular hypertrophy	Yes	3
Dislocation of ulna	Yes	5

A score >7 has a ~85% accuracy for predicting an *SHOX* genetic abnormality.

Tall Stature

Tall stature can be defined as a height more than 2 SD above the population mean for that child's age, gender and ethnic origin. While short stature remains a common reason for referral to a pediatric endocrinologist, there are far fewer children who present because of tall stature, presumably because being tall is generally more socially acceptable than having restricted growth. They are mostly girls. Causes of tall stature are outlined in Table 6.6. Most children with tall stature have a benign

Table 6.6 Aetiology of tall stature.

Familial tall stature
Constitutional tall stature
Obesity
Endocrinopathy
Precocious puberty
Hyperthyroidism
Familial glucocorticoid deficiency
GH excess – gigantism/acromegaly
Genetic syndromes
Beckwith–Wiedemann
Homocystinuria
Klinefelter (XXY)
Marfan syndrome
Simpson–Golabi–Behmel
Sotos
Weaver
XXX
XYY

cause for their growth acceleration – familial tall stature, constitutional tall stature or obesity.

Familial Tall Stature, Constitutional Tall Stature and Obesity

Children with familial tall stature are usually tall from an early age (2 years of age or younger) with one or more parents who are also tall. They have a high normal growth rate and bone age consistent with chronological age. Clinical examination and evaluation of parental heights are all that is required to confirm the diagnosis. If there is one tall parent and one normally statured parent, consideration should be made of the possibility of an autosomal dominant condition (such as Marfan syndrome) before making a diagnosis of familial tall stature.

Constitutional tall stature can be considered a disorder of the tempo of growth. Children are born of normal stature and grow rapidly during the first 4 years of life; after which growth velocity drops and becomes parallel to the 50th centile. Bone age may be slightly advanced. Puberty is usually within the early normal range and final height usually within the target range.

Children with obesity from early childhood are usually tall with a slightly advanced bone age. Puberty often starts earlier than average, and final adult height is increased only slightly compared with mid-parental height.

Precocious Puberty

Although precocious puberty results in a reduction in final height due to restricting the number of years of prepubertal growth, at the time of onset of puberty, height is generally increased. Therapy should be directed at the underlying pubertal disorder and most commonly involves GnRH analogue therapy for gonadotropin-dependent precocious puberty that decreases sex steroid production and IGF-I concentrations, thereby reducing growth rate.

GH Excess

GH excess is extremely rare. Causes include GH-secreting pituitary micro- or macroadenomas, ectopic GHRH production and genetic abnormalities affecting GH secretion (McCune–Albright syndrome and Carney complex). The commonest symptom is rapid growth. Acromegalic features, such as soft tissue growth of the hands and feet, mandibular overgrowth with prognathism, forehead protrusion and deepening of voice, can also occur but are rare in children. The presence of acromegalic features is likely to be linked to the timing of onset (more common with onset in adolescence). Other clinical features may include excessive sweating, carpal tunnel syndrome, lethargy, arthropathy, impaired glucose tolerance and hypertension.

Diagnosis of GH excess is based upon the clinical features, auxology and biochemical evidence. First line investigation should be measurement of IGF-I concentration compared to normal ranges for age, gender and pubertal status. Puberty-specific ranges are important as children with an early puberty frequently present with tall stature and have high IGF-I concentrations for age but IGF-I concentrations fall into the normal range for pubertal stage. A random GH concentration cannot confirm the diagnosis of GH excess but if <0.4 μg/L, the diagnosis of GH excess is excluded. Standard investigations include an oral glucose tolerance test for GH suppression and GH day curves. A GH day curve involves measurement of at least five separate GH concentrations over 12 hours but, given that maximal physiological GH secretion occurs in adolescence and there are no normative data, interpretation of this test can be challenging. During an OGTT, GH concentrations should fall to ≤0.4 μg/L.

Benign GH-secreting adenomas are the most common cause of GH excess. In children and adolescents, it is important to examine for genetic mutations predisposing to such tumours and sequencing of genes encoding MENIN, p27 and the *AIP* should be undertaken. Trivellin et al. identified heritable microduplications on chromosome Xq26.3 in 13 patients (9 female) with early childhood-onset pituitary gigantism, X-linked acrogigantism (X-LAG) [17]. One of three genes in the critical duplicated region, *GPR101*, encodes an orphan G-protein-coupled receptor and is likely responsible for the phenotype. The expression of *GPR101* was greatly increased in the pituitaries of those patients with the duplication. Additionally, *GPR101* mutations were detected in 11/248 patients with sporadic acromegaly, supporting a role in the aetiology of pituitary gigantism/acromegaly.

McCune–Albright syndrome causes GH excess as the GHRH receptor is a G-protein-coupled receptor. The constitutively active alpha stimulatory G-protein subunit in McCune–Albright leads to overactivity of GHRHR signal transduction and GH excess. Patients with McCune–Albright syndrome are predisposed to the development of polyostotic fibrous dysplasia and GH excess may contribute to the enlargement of such lesions. Reports of the incidence of GH excess in McCune–Albright are limited but suggest around 20% have GH excess both with and without visible adenomas on MRI of the pituitary.

The Carney complex is an autosomal dominant disorder characterized by skin pigmentary abnormalities, myxomas, schwannomas and endocrine tumours or

overactivity. It is caused by loss-of-function mutations in the *PRKAR1A* gene that encodes the regulatory subunit of protein kinase A. Dissociation of the regulatory subunits from the catalytic subunits of protein kinase A leads to activation of signal transduction. Under normal circumstances this dissociation is triggered by cAMP. Carney complex-associated mutations lead to loss of the regulatory subunit and increased activity of protein kinase A-associated signal transduction. GH-secreting adenomas are reported in 10% of adults with Carney complex. These are rare before puberty but there is likely to be a prolonged period of GH hypersecretion before the development of true GH excess. Hyperprolactinaemia is common in patients with Carney complex and GH excess.

Treatment of GH excess is with transsphenoidal surgery, medical treatment and/or radiotherapy. Surgery is the treatment of choice for microadenomas, for macroadenomas without cavernous sinus involvement and for tumours causing symptoms from compression. A biochemical cure is seen in 75–95% of patients.

Medications used to treat GH excess include the somatostatin analogues (octreotide, lanreotide) and the GHR antagonist pegvisomant. Medical therapy is used where the tumour is not suitable for surgery, where surgery/radiotherapy has failed to achieve a biochemical cure, or for patients who are not fit for surgery due to comorbidities. Somatostatin analogues are effective in reducing GH and IGF-I concentrations as well as shrinking tumour size, achieving biochemical cure in 70% and tumour shrinkage in 75%. Octreotide requires multiple daily injections but the longer-acting somatostatin analogues require only once-monthly injections. Pegvisomant is more effective at reducing IGF-I concentrations but GH concentrations can no longer be used to monitor the disease during therapy. Unlike the somatostatin analogues it does not lead to tumour shrinkage.

Dopamine agonists such as cabergoline have been used to treat acromegaly but are less effective than the other medical treatments and may be best used where tumours co-secrete GH and prolactin.

Radiotherapy is generally reserved as a third-line treatment due to the time taken to achieve maximum effect (up to 10 years) and risks of hypopituitarism (up to 50% by 5 years post-radiotherapy), visual problems and late effects of cerebrovascular disease and second tumours.

Syndromes Associated with Tall Stature

Klinefelter Syndrome and 47,XYY

With an incidence of 1 in 500 newborn boys, Klinefelter syndrome is the commonest sex chromosomal abnormality. Ninety percent of affected individuals have the karyotype 47,XXY with 10% having some degree of 47,XXY/46,XY mosaicism. In childhood, the presentation is with tall stature and/or poor virilization at puberty but not with a delayed onset. By contrast, in adulthood, the usual presentation is with infertility. The legs are long and arm span is usually greater than height. A rapid increase in growth velocity is seen during the childhood period but the adolescent growth spurt is not of increased magnitude. Thus boys with Klinefelter syndrome who are normally statured at the beginning of adolescence can be reassured that their final height will not be increased. Adult height is usually slightly greater than the mid-parental height.

This is in contrast to males with 47 XYY karyotype (1 in 1000 males) who display both an increased childhood growth velocity and an increase in the magnitude of the adolescent growth spurt. Consequently adult height in XYY can be a mean of 13 cm above the mid-parental height. Affected individuals are not dysmorphic. There is an increased risk of learning difficulties, developmental delay, hypotonia and behavioural and emotional problems.

Other clinical features suggestive of Klinefelter syndrome include gynaecomastia, decreased muscle mass, increased body fat, cryptorchidism, mild learning difficulties and behavioural difficulties. Onset of puberty is not delayed but the testes do not enlarge beyond 8–10 mL. The diagnosis is made by examining the karyotype. Typically the gonadotropins will be raised although this will not be present until after the onset of puberty. Serum testosterone concentrations may be within the normal range. Often the concentration of sex hormone-binding globulin is raised and consequently the free testosterone is low.

The phenotype is relatively mild compared with other conditions where there is gain or loss of one of the autosomes. This is likely to be due to inactivation of most genes on the extra X chromosome. Genes in the pseudoautosomal region probably escape inactivation and lead to the observed phenotype. The extra copy of the SHOX gene in the pseudoautosomal region is likely to lead to the increased height seen in Klinefelter syndrome.

Other Tall Stature Syndromes

Marfan syndrome is an autosomal dominant multisystem connective tissue disease caused by mutations in the fibrillin 1 gene. The incidence is ~1 in 5000. Diagnosis is via the Ghent criteria (Tables 6.7 and 6.8).

Homocystinuria is an autosomal recessive trait caused by loss-of-function mutations in the gene encoding cystathionine beta-synthase. Affected individuals are tall and thin with increased arm span. Other clinical features shared with Marfan syndrome include pectus deformities, myopia and scoliosis. Although patients with homocystinuria are also affected by lens dislocation, typically

Table 6.7 Criteria for diagnosis of Marfan syndrome.

In the absence of a family history
1) Aortic dilatation (*Z* score ≥2) and ectopia lentis = Marfan syndrome
2) Aortic dilatation (*Z* score ≥2) and fibrillin 1 mutation = Marfan syndrome
3) Aortic dilatation (*Z* score ≥2) and systemic score ≥7 = Marfan syndrome
4) Ectopia lentis and fibrillin 1 mutation identified that has previously been associated with aortic dilatation = Marfan syndrome
In the presence of a family history
1) Ectopia lentis and a family history of Marfan syndrome = Marfan syndrome
2) Systemic score ≥7 and family history of Marfan syndrome = Marfan syndrome
3) Aortic dilatation (*Z* score ≥2 for those >20 years of age or *Z* score ≥3 in patients aged <20 years) and family history of Marfan syndrome = Marfan syndrome

Table 6.8 Systemic scoring system.

• Wrist AND thumb sign – 3 (wrist OR thumb sign – 1)
• Pectus carinatum deformity – 2 (pectus excavatum or chest asymmetry – 1)
• Hindfoot deformity – 2 (plain pes planus – 1)
• Pneumothorax – 2
• Dural ectasia – 2
• Protrusio acetabuli – 2
• Reduced upper segment/lower segment ratio AND increased arm/height ratio AND no severe scoliosis – 1
• Scoliosis or thoracolumbar kyphosis – 1
• Reduced elbow extension – 1
• Facial features (3/5) – 1 (dolichocephaly, enophthalmos, down-slanting palpebral fissures, malar hypoplasia, retrognathia).
• Skin striae – 1.
• Myopia > 3 diopters – 1.
• Mitral valve prolapse (all types) – 1.

this is downwards. In contrast to Marfan syndrome, intellectual disability is common, aortic aneurysms are uncommon and thrombosis and osteoporosis are common. Diagnosis is made with examination of serum and urine amino acids.

Sotos syndrome (also called cerebral gigantism) is a sporadic disorder caused by mutations or deletions in the *NSD1* gene. It is characterized by macrocephaly, dysmorphic facial features (high broad forehead, long narrow face, down-slanting palpebral fissures, prominent jaw), poor coordination and learning difficulty. Height velocity is increased during the first 4 years of life and thereafter reduces to an age-appropriate height velocity.

Weaver syndrome is phenotypically distinct from Sotos syndrome with hypotonia, looseness of skin, wide philtrum, deep-set nails and advanced bone age (not found in Sotos syndrome) and is caused by autosomal dominant mutations in *EZH2*.

Simpson–Golabi–Behmel syndrome is an X-linked disorder caused by mutations in the gene encoding glypican 3 (GPC3) and results in pre- and postnatal overgrowth with coarse facial features, macrosomia, macroglossia, postaxial polydactyly, neonatal hypoglycaemia, talipes, pectus excavatum and an increased risk of neoplasms. Intelligence is either normal or only mildly impaired.

Beckwith–Wiedemann syndrome is characterized by overgrowth including visceromegaly and hemihypertrophy, ear lobe creases, hypoglycaemia, omphalocele and an increased risk of tumours. The genetic causes of Beckwith–Wiedemann syndrome are complex and include paternal uniparental disomy of chromosome 11 and hypermethylation at the H19 differentially methylated region, both of which lead to increased expression of IGF-II. It can also be caused by mutations in the maternal inherited copy of CDKN1C gene or hypomethylation at KvDMR1. Both processes lead to a reduction in concentrations of the growth-suppressing protein $p57^{kip2}$.

Management of Tall Stature

There is usually no medical reason for treatment of tall stature and the decision to treat is based upon the acceptability for the patient and parents of the predicted final height. In boys the treatment has traditionally been the use of intramuscular injections of testosterone esters given at supraphysiological doses – up to 500 mg/m^2/month (given as 1–2 injections per month). This equates to ~10 times natural testosterone production in early adolescence or 4 times the testosterone production of adult men. Results have been variable with small initial studies suggesting a reduction of 4.5–7.5 cm in final height. More recent studies have suggested a more modest reduction in final height with greater reductions in height achieved when the bone age is younger at start of treatment. Where treatment was started at a bone age of 14 years or above, it resulted in an *increase* in final height; thus treatment should generally be avoided in boys where the bone age is 14 years or greater.

Testosterone treatment increases prevalence of acne but does not result in increased aggressive or sexualized behaviours outside the normal range for adolescence. During treatment, high-dose testosterone suppresses gonadotropin secretion, testicular size and spermatogenesis. In adults who previously received treatment, however, testicular volumes, gonadotropin concentrations,

testosterone concentrations and sperm quality are within normal limits.

In girls high-dose oestrogen treatment was most commonly given as oral ethinylestradiol at 100 µg/day (physiological adult replacement is equivalent to 30 µg/day) in combination with an oral progesterone for 7–10 days per month. Mean reductions in height of up to 6 cm were observed and similarly to boys, treatment was more effective when started at a lower bone age. No significant effect on height reduction was observed when treatment is started when bone age is > 13.5 years [18]. Common side effects of high-dose oestrogen therapy included nausea, headache, weight gain and vaginal discharge. Lifetime oestrogen exposure is linked to breast cancer risk and emerging evidence suggests that high-dose oestrogen therapy is associated with an increased risk of malignancy. Of additional concern, women treated with 100–200 µg/day ethinylestradiol also had a dose-dependent reduction of fertility in adulthood. Consequently this approach cannot be recommended. Early induction of puberty with physiological doses of testosterone in boys and oestrogen in girls may be beneficial, without the additional risks of high-dose oestrogen therapy.

Surgery to limit growth is possible via a bilateral epiphysiodesis of both femora and tibiae. Surgery is effective and low risk but there remain concerns about the very rare but serious adverse effects including osteomyelitis and limb loss in what is essentially a benign condition.

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7

Puberty and Its Disorders

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KEY LEARNING POINTS

- Puberty is the developmental stage when reproductive capacity is attained. The changes that occur are dependent on events in fetal life and early infancy. The trigger for the reactivation of the hypothalamic–pituitary–gonadal axis at the onset of puberty is a focus of much interest but the understanding of the key elements that control these events is limited.
- The timing of puberty has a near-normal distribution in the general population, with the definitions of significantly early or delayed puberty being statistically defined. Pubertal timing not only is strongly determined by genetics but also depends on environmental factors such as BMI, nutrition, psychosocial factors and endocrine-disrupting chemicals. It is important for clinicians to distinguish benign variants of puberty and cases with an underlying pathological basis.
- Precocious puberty may be central or peripheral in origin. A knowledge of the different clinical forms of precocious puberty is essential to determine whether there is a tumour (intracranial, gonadal or adrenal) or other disease (neurofibromatosis, McCune–Albright syndrome, congenital adrenal hyperplasia) and the indications for treatment or observation. The psychological aspects of precocious puberty should be evaluated during treatment of these patients.
- Constitutional delay of puberty is the single most common cause of delayed puberty in both sexes but it can be diagnosed only after underlying conditions have been excluded. Important differential diagnoses include congenital or acquired hypogonadotropic hypogonadism, chronic disease and primary hypogonadism. It is important to diagnose the underlying cause of delayed puberty, especially to distinguish between self-limited delayed puberty and hypogonadotropic hypogonadism in adolescents, as treatment aims, options and duration are very different in the two groups.
- Mini-puberty is an important window of opportunity for the evaluation of suspected hypogonadism in an infant and diagnosis during the mini-puberty may aid management and future outcomes.
- The consideration of fertility in patients with hypogonadism, even in adolescence, should be paramount for clinicians as appropriate treatment may optimize fertility potential in a time-sensitive manner that may not be possible later in life. There is a need for awareness of the clinical spectrum of hypogonadotropic hypogonadism and the differing requirements of patients with severe congenital hypogonadotropic hypogonadism versus partial or indeed reversible hypogonadotropic hypogonadism.

Normal Puberty

Introduction

Puberty results in the achievement of adult height and body proportions as well as development of the genitalia and the capacity to reproduce. The activation of pulsatile hypothalamic gonadotropin-releasing hormone (GnRH) secretion from the hypothalamus is the endocrine hallmark of the onset of puberty. This activation results in increased luteinizing hormone (LH) and follicle-stimulating hormone (FSH) release from the anterior pituitary, which act on the gonads to stimulate their development, gametogenesis and sex steroid and gonadal peptide hormone production (Figure 7.1).

Clinical features of puberty include the appearance of secondary sexual characteristics, acceleration of the growth rate and an increase in bone mineral mass and body mass index (BMI). All these changes are the consequence of the increase of sex hormone synthesis by the gonads under the control of the hypothalamic–pituitary axis. Hypothalamic neurons secreting GnRH are at the heart of a complex neuroendocrine network that controls the synthesis of sex hormones. This network is

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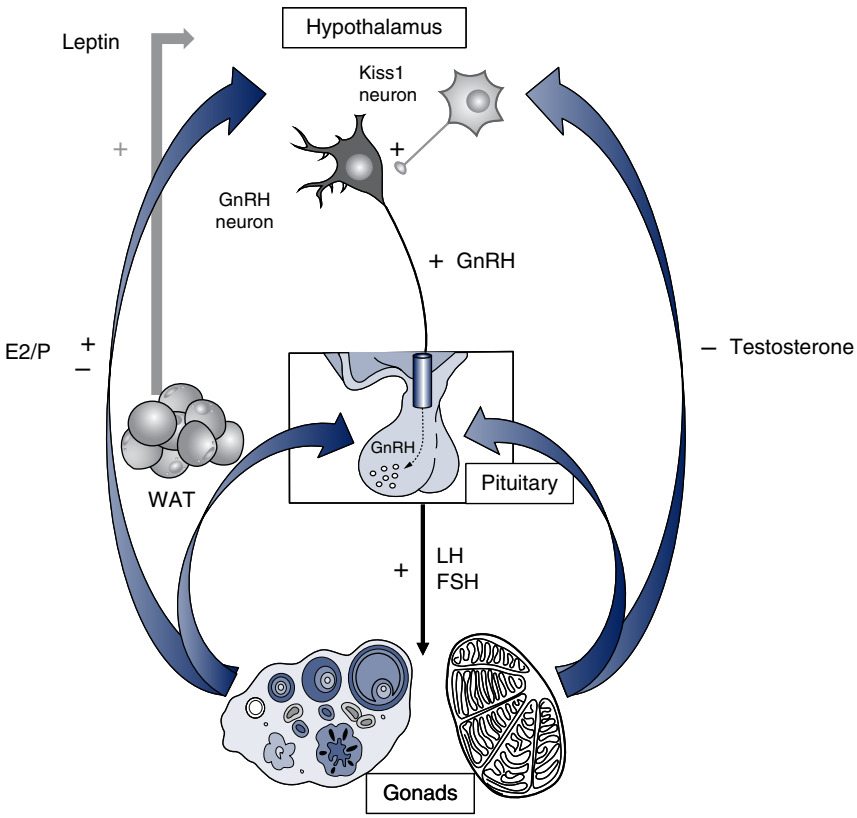

Figure 7.1 Schematic of the hypothalamic–pituitary–gonadal axis. WAT, white adipose tissue; E2/P, oestradiol, progesterone.

composed of kisspeptins, neurokinin B and dynorphin-expressing neurons as well as glial cells such as tanycytes, astrocytes and ependymal cells. These neurons and glial cells act together to regulate the pulsatile secretion of GnRH.

The development of the hypothalamic–pituitary axis starts in fetal life and continues until the acquisition of reproductive capacity. After the axis is active in the fetus, it is reactivated in early infant life, the so-called 'mini-puberty', and then becomes dormant with concentrations of LH and FSH low or undetectable in young children between the ages of 2 and 8–9 years. Development of the clinical features of puberty is initiated by the reactivation of the hypothalamic–pituitary–gonadal (HPG) axis after the quiescence during childhood. What drives suppression of the axis during childhood and controls the release of this brake and its timing is little understood; however, advances in the understanding of this chronology have been described in recent years, thanks to the study of rare pubertal diseases and relevant animal models, predominantly in mice.

Physical Changes that Occur During Puberty

In boys the first physical sign of puberty is an increase in testicular volume above the prepubertal volume of 3 mL (Tanner stage G2) [1] (Figure 7.2), which can be assessed using the Prader orchidometer or ruler. In girls the most common first physical sign is breast development (Tanner stage B2) [3] (Figure 7.2). There is a classic pattern of pubertal progression in both sexes, with breast and pubic hair development in girls and testicular enlargement followed by pubic hair development and penile growth in boys (Table 7.1). The physical changes are classified by the definitions described by Tanner and include five stages for breast, male genital and pubic hair development and three stages for axillary hair development. A simplified classification system has more recently been described for clinicians less familiar with assessing Tanner stage, with only three categories: prepubertal (equivalent to Tanner stage 1), pubertal (Tanner stages 2–3) and complete puberty (stages 4–5).

Although pubic and axillary hair development is primarily governed by adrenal androgens, the stage of breast or testicular maturation is normally equal or near equal to pubic hair staging. Breast enlargement in girls may be unilateral for several months and may continue to be asymmetrical. Menarche in girls occurs as breast and pubic hair development are near adult stage. The first 2 years of menses are anovulatory in 55–99% of cycles, and it is not until 5 years after menarche that 80% of cycles are ovulatory. Anovulatory periods may develop before secondary sexual development is complete and girls who have not yet reached adult Tanner stages may

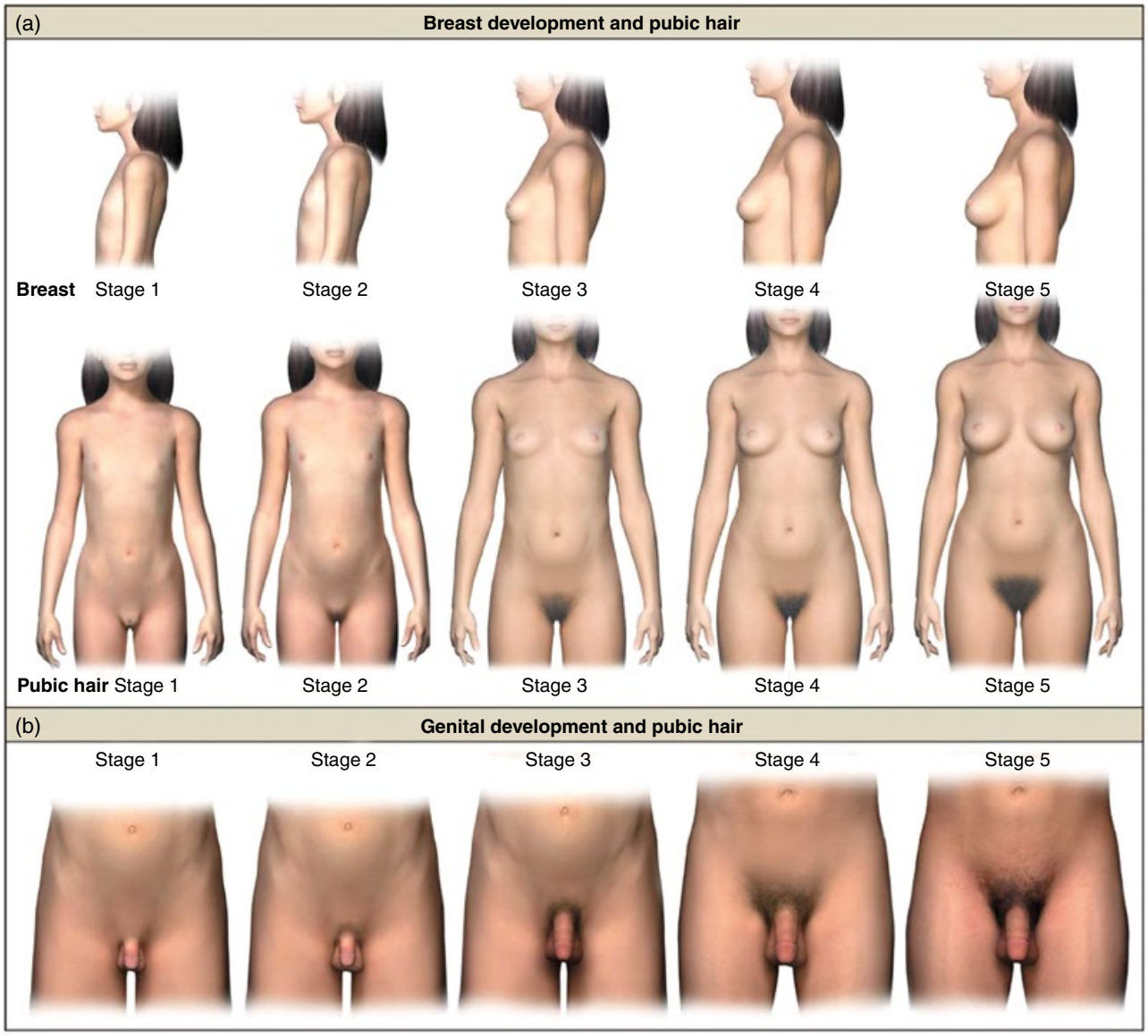

Figure 7.2 Tanner staging of puberty onset in boys and girls. *Source:* From Carel and Leger [2], reproduced with author permission.

Table 7.1 Details of the Tanner stages of puberty.

Tanner stage	Females		Males	
	Breast	Pubic hair	Genitalia	Pubic hair
1	Nipple elevation only	None	Testis, 1–3 mL	None
2	Small raised breast bud	Growth along labia, sparse, lightly pigmented	Testis, >3 mL Scrotal enlargement	Sparse, lightly pigmented
3	Breast and areola enlarge with no contour difference	Increases in amounts, darkens, starts to curl	Testis continues to enlarge, penis lengthens	Increases in amounts, darkens, starts to curl
4	Further enlargement with nipple and areola projecting to form secondary mound	Resembles adult type but not spread to medial thighs	Scrotum darkens, widening of glans penis	Resembles adult type but not spread to medial thighs
5	Adult contour with areola and breast in same contour, nipple projecting	Spreads to medial thighs, adult distribution	Adult size and morphology	Spreads to medial thighs, adult distribution

have the capacity for fertility. Voice changes in boys can be noted at 8 mL testicular volume and become obvious by 12 mL volume. The tempo and process of puberty are well conserved across ethnicities and populations.

Ovarian, Uterine and Vaginal Development in Puberty

Oogonia arise from primordial germ cells in the wall of the yolk sac near the caudal end of the embryo. By the sixth month of fetal life, the cells have migrated to the genital ridge and progressed through sufficient mitoses to reach a complement of 6–7 million oogonia, which represents the maximal number of primordial follicles the individual will have throughout life. Meiosis begins but is not completed before pubertal maturation of the ovaries, as the nucleus and chromosomes persist in prophase to mark the conversion of the oogonia to primary oocytes. Primordial follicles are composed of the primary oocyte surrounded by a single layer of spindle-shaped cells that will develop into granulosa cells and a basal lamina, which will be the boundary of the theca cells later in development. Due to apoptosis, 2–4 million primordial follicles are left at birth but only 400,000 remain at the onset of menarche.

At the time of the first ovulation, the first meiotic metaphase converts the primary oocyte into the secondary oocyte, which is extruded into the fallopian tubes. The ovum does not form until the time of sperm penetration when the second polar body is eliminated. While some follicles in the fetus and child progress to the large antral stage, all developing follicles undergo atresia prior to puberty and few large follicles develop in the child. The presence of more than six follicles with a diameter of >4 mm indicates the presence of pulsatile gonadotropin secretion and may be seen in normal prepubertal girls, in pubertal girls before menarche and in patients recovering from anorexia nervosa. This 'multicystic' appearance is considered characteristic of a phase of mainly nocturnal pulsatile gonadotropin secretion prior to positive feedback.

Standards for ovarian and uterine size and shape are available for normal girls and those with Turner syndrome. The uterus lies in a craniocaudal direction in childhood without the flexion seen in adults. The myometrium enlarges during early puberty, thereby enlarging the corpus leading to the adult corpus to cervix ratio. The cervix develops its adult shape and size just before menarche and the cervical canal enlarges. Assessment of endometrial thickness is a useful monitoring tool during pubertal development as a marker of oestrogenic action.

The vagina lengthens early in puberty and continues to elongate at least until menarche. The vulval and vaginal mucosa softens and thickens, with a colour change from red to pink, and the hymen thickens and its orifice enlarges. The mons pubis enlarges and labia becomes larger and wrinkled. There is only slight clitoral enlargement during puberty and any noticeable virilization suggests underlying pathology.

Testicular Development in Puberty

The prepubertal testes consist mainly of Sertoli cells, some haploid spermatogonia and interstitial cells, whereas testes volume is mostly determined by germ cells in the seminiferous tubules in the adult. The seminiferous tubules enlarge in diameter and elongate during puberty forming tight occlusive junctions leading to the development of the blood–testis barrier. Leydig cells are present in small numbers in prepuberty, although the interstitial tissue is mainly composed of mesenchymal tissue. At puberty, the Leydig cells become more apparent. Penile and testicular growth usually correlates with pubic hair development.

Spermatogenesis can be detected histologically between ages 11 and 15 years and sperm is found in early morning urine samples on average at 13.3 years of age (spermarche). First ejaculation occurs at a mean age of 13.5 years without a consistent relationship to testicular volume, pubic hair development or phallic enlargement. While adult morphology, motility and concentration of sperm are not found until the bone age advances to 17 years, immature-appearing boys can be fertile.

Voice Break

Voice breaking in boys occurs due to the increased length of the vocal cords that follows the growth spurt of the larynx. It can be used as a marker of late puberty in boys, with over 30% of boys completing voice break by 14 years. Self-recall of timing of voice breaking has been used for assessment of timing of puberty in large population studies to parallel the self-recall of age of menarche in females. Changes in voice and facial appearance with facial hair development and jaw growth continue after secondary sexual development has been completed.

Gynaecomastia

Breast enlargement occurs to some degree after the early stages of puberty in 39–75% of boys. It has been proposed, albeit without clear evidence, that pubertal gynaecomastia involves a relative imbalance between free oestrogen and free androgen actions in the breast tissue, which can occur through multiple mechanisms. During mid-to-late puberty, a relatively higher ratio of oestrogen to testosterone generated by the testes and peripheral tissues leads to physiological gynaecomastia before testosterone secretion reaches adult concentrations.

It is important to distinguish idiopathic pubertal gynaecomastia from underlying pathology. Pathological causes include secretion of too much oestradiol from a

Leydig cell, Sertoli cell or adrenal tumour, hyperthyroidism, under-secretion of testosterone in Klinefelter syndrome, androgen insensitivity and ovotesticular disorders. Other associations include obesity, liver disease, radiation exposure and other causes of hypogonadism. Autosomal dominant mutations in the aromatase (*CYP19A1*) gene result in constitutively increased gene transcription and overexpression of the aromatase enzyme leading to an increased oestrogen-to-androgen ratio. Gynaecomastia may be secondary to medications including spironolactone, cimetidine, ketoconazole, oestrogens, anti-androgens, growth hormone (GH), GnRH analogues, 5α-reductase inhibitors, tricyclic antidepressants, chemotherapeutic agents, cardiovascular medications and drugs of abuse including marijuana, ethanol, heroin and amphetamines. Exposure to oestrogen-containing substances such as lavender and tea tree oils and phytoestrogens has also been associated with this condition. Differential diagnosis of male breast enlargement also includes lipomastia, pseudogynaecomastia and, very rarely, lipoma or neurofibroma. Gynaecomastia in a prepubertal boy is pathological and warrants investigation.

Physiological gynaecomastia typically resolves as puberty progresses and testosterone concentrations increase; gynaecomastia in a mid-pubertal boy who is otherwise healthy does not warrant investigation. Treatment of the underlying disorder or removal of the exogenous cause is appropriate if identified. In most cases the tissue regresses within 2 years but occasionally in normal boys (often with increased BMI) and frequently in pathological conditions such as Klinefelter syndrome or partial androgen resistance where the effective amount of bioactive testosterone is reduced, gynaecomastia persists. Surgery, usually through a peri-areolar incision, is the only effective treatment at present. Although non-aromatizable androgens, anti-oestrogens or aromatase inhibitors (AIs) are being used to some extent, none of these treatment modalities have shown efficacy different from placebo in controlled clinical trials [4].

Bone Mineral Density

The most important phases of bone accretion occur during infancy and puberty. In the teenage years, girls reach peak mineralization between 14 and 16 years while boys reach a peak at 17.5 years. Both peaks are attained after peak height velocity (PHV) has been achieved. Bone mineral density is influenced not only by sex steroids but also by genetic factors, nutrition, exercise (particularly high impact) and the action of GH.

There is a poor correlation between calcium intake and BMD during puberty or young adulthood, suggesting that the timing of puberty is the most significant factor in achieving peak bone mineralization. It seems prudent nonetheless to ensure adequate calcium intake in patients with delayed or absent puberty or those treated with GnRH analogues to hold up puberty until more is learned about the biology of the control of bone accretion in puberty.

Body Composition

Percentages of lean body mass, skeletal mass and body fat are equal between prepubertal boys and girls but, as boys go through puberty, total body bone mass and fat-free mass continue to increase while in girls only body fat and fat-free mass increase. The increase in lean body mass starts at 6 years in girls and 9.5 years in boys and is the earliest change in body composition in puberty. At maturity, men have 1.5 times the lean body mass and almost 1.5 times the skeletal mass of women, while women have twice as much body fat as men. In males the shoulders become broader and in females the hips become wider during puberty.

Timing of Puberty and Relationship with Linear Growth

Timing of puberty

In the general population, there is a near-normal distribution of the timing of puberty, with the mean age of onset of G2 at 11.5 years in boys (Figure 7.3) and 11 years for B2 in girls (Figure 7.3). Within this distribution there has been in recent years an increasing degree of skewness at both ends of the spectrum, as an earlier age of pubertal onset (B2 or G2) has become more prevalent, as well as an increase in the number of children completing their puberty at a later age [6].

In healthy boys, the normal age limits for G2 development are 9–14 years. Most Caucasian girls have at least early signs of secondary sexual development by 13 years of age (Figure 7.3). While a large variability in the timing of pubertal onset exists in both genders, clear age cut-offs for normal pubertal development have been drawn but the age limits for identifying children who need evaluation for precocious or delayed puberty (DP) may vary in different ethnic groups.

The puberty growth spurt encompasses the most rapid phase of growth after the neonatal period and follows the decreasing growth rate of the late childhood phase. PHV and peak pubertal GH production coincide approximately with midpoint of pubertal development (Figures 7.4 and 7.5). Up to 25% of total adult height is achieved from growth during puberty but the amplitude and peak velocity of the pubertal growth spurt is not fixed and varies with age at onset of puberty.

Clear gender discrepancy exists in both the age at and degree of PHV attained. In girls, PHV occurs at B2–3 at

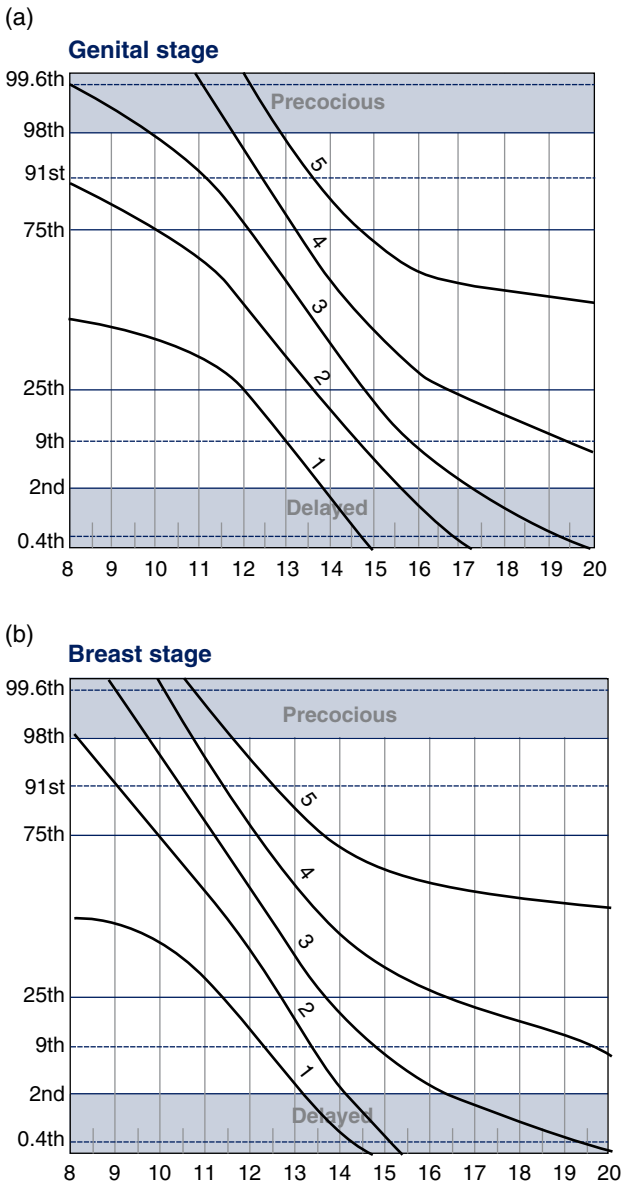

Figure 7.3 The distribution of pubertal timing in healthy boys (a) and girls (b). These data have been incorporated into the UK growth charts and are available at www.growthcharts.rcpch.ac.uk. The lines 1–5 correspond to Tanner stages 1–5, *x*-axis values are age in years and *y*-axis gives the cut-off for gender appropriate centiles for development. Individuals can have their Tanner stage plotted to determine precocity, delay in development or development that has plateaued or arrested. *Source:* Original data from van Buuren [5].

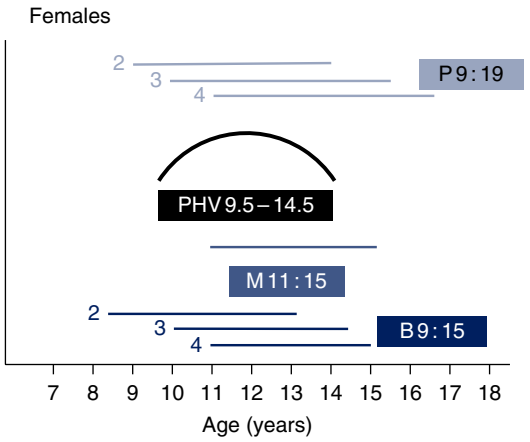

Figure 7.4 Relationship between peak height velocity and pubertal development (female). P, pubic hair stage; PHV, peak height velocity; M, menarche; B, breast stage. Age ranges presented: in coloured boxes – 3rd–97th centiles; in linear format – 3rd–97th centiles for each tanner stage, e.g. B2. Curved lines represent pubertal growth spurt with age ranges for PHV. *Source:* Original concept from Tanner [7]. Data from van Buuren [5] and Lawaetz et al. [8].

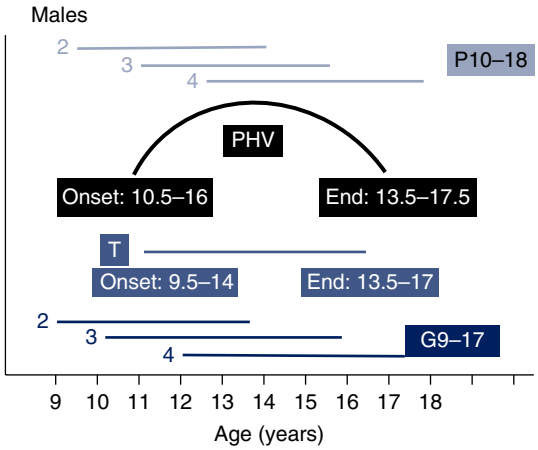

Figure 7.5 Relationship between peak height velocity and pubertal development (male). P, pubic hair stage; PHV, peak height velocity; T, testicular growth; G, genital stage. Age ranges presented: in linear format – 3rd–97th centiles for each Tanner stage, e.g. G2; curved lines represent pubertal growth spurt with age ranges for onset and completion; in coloured boxes – age range in years for onset to completion of each parameter. *Source:* Original concept from Tanner [7]. Data from van Buuren [5] and Lawaetz et al. [8].

an average age of 11.5 years with a PHV of 8.3 cm/year. In boys PHV coincides with G3–4 at an average age of 13.5 years and achieves an incremental rate of 9.5 cm/year. The mean difference in adult height between men and women of 12.5 cm is due mainly to the taller stature of boys at the onset of the pubertal growth spurt and also to the increased height gained during the pubertal growth spurt in boys compared with girls. A girl who has experienced menarche usually has no more than 2–3% of her growth remaining, an average of 5–7.5 cm before adult height is reached, although the range of post-menarcheal growth extends to 11 cm.

The effect of the timing of puberty on pubertal growth is measurable: early puberty is associated with a large pubertal growth spurt while late maturers, who have a

longer prepubertal period of growth, experience a less pronounced pubertal growth spurt. Therefore, there is regulatory compensation at work: although extremes of pubertal timing potentially lead to a degree of final height reduction due to the reduced overall period of growth in precocious puberty (PP) or poor PHV in DP, the timing of puberty does not greatly influence adult height.

Sex steroids exert a direct effect on growing cartilage as well as an indirect effect mediated by increasing GH secretion. Increasing sex steroid production at puberty stimulates increased amplitude (but not frequency) of spontaneous GH secretion as well as peak-stimulated GH and this in turn stimulates increased production of insulin-like growth factor 1 (IGF-1). IGF-1 concentrations rise gradually throughout childhood but increase several fold at the time of the pubertal growth spurt. Oestrogen, either from the ovary or aromatized from testicular testosterone, is the factor that mediates the increased GH response during puberty. A prepubertal child given an androgen that can be aromatized to oestrogen, such as testosterone, will have augmented GH secretion, whereas non-aromatizable dihydrotestosterone will not increase GH secretion. An oestrogen-blocking agent such as tamoxifen will reduce GH secretion (Figure 7.6).

Oestrogen has a biphasic effect on growth: low concentrations stimulate growth while higher concentrations lead to its cessation. Oestrogen plays a major role in the final stages of epiphyseal fusion. Patients with oestrogen receptor deficiency or aromatase deficiency have tall stature, continued growth into the third decade due to the lack of fusion of the epiphyses of the long bones, increased bone turnover, reduced bone mineral density, osteoporosis and the absence of a pubertal growth spurt. Thus oestrogen is the main factor that fuses the epiphyses of the long bones and causes cessation of linear growth. These observations have led to the use of AIs for the further management of short stature associated with a variety of conditions, with the rationale that this might allow more time for growth prior to epiphyseal fusion.

Thyroid hormone is necessary in sufficient amounts to allow the pubertal growth spurt to proceed. The rapid growth rate is accompanied by an increase in markers of bone turnover such as serum alkaline phosphatase, serum bone alkaline phosphatase, osteocalcin, Gla protein and the amino-terminal propeptide of type III procollagen; normal adult values of these proteins are lower than concentrations found in puberty.

Regulators of the Timing of Puberty

The variability in the timing of puberty in healthy adolescents is governed by complex regulatory mechanisms including genetic, environmental and other factors. Nutritional status, adoption, geographical migration and emotional well-being all have an effect on pubertal timing. The timing of puberty in most countries in the developed world exhibited a rapid decrease in the first half of the 20th century, most notably in girls [9] (Figure 7.7). These trends have been less clearly shown in

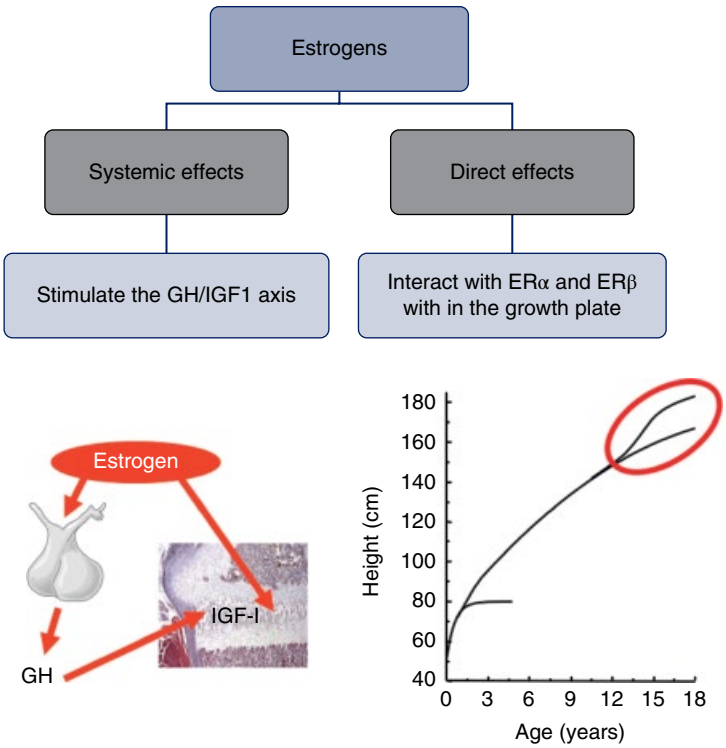

Figure 7.6 Effects of oestrogen on growth. Oestrogens exert systemic effects not only via the growth hormone/IGF1 axis but also locally within the growth plate. Growth chart shows the difference between male and female height attainment during the pubertal growth spurt.

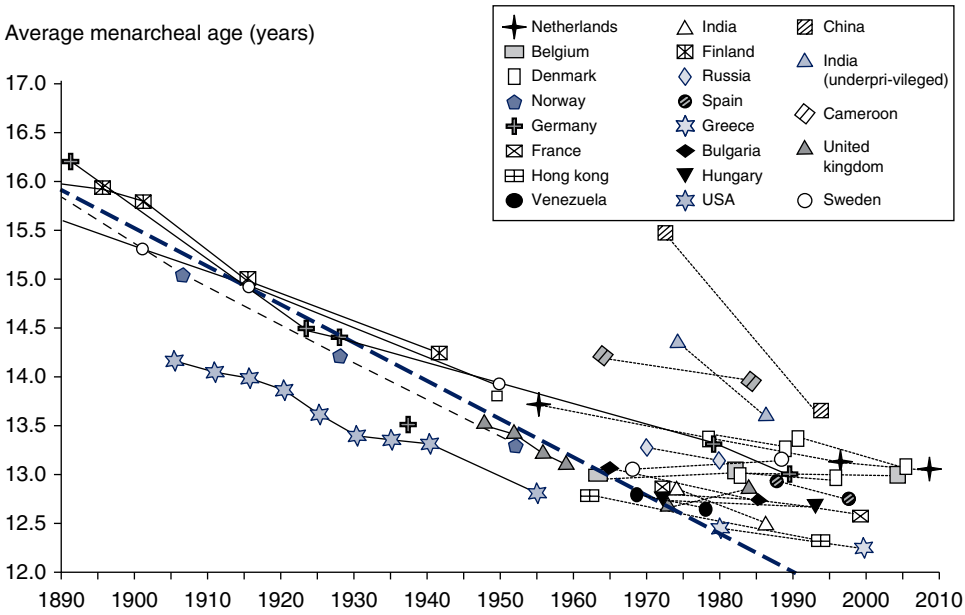

Figure 7.7 Evolution of average menarcheal age (year) in the USA and Nordic countries between 1890 and 1960. *Source:* From Parent et al. [6], reproduced with permission of Oxford University Press.

boys. A small but significant change in the normal spectrum of timing of G2 development has been documented in a European cohort and in the USA, but remains controversial.

Much has been postulated about this observed secular trend towards an earlier age of pubertal onset in the developed world. Nutritional changes clearly have an important role, as shown by the positive correlation between age at puberty onset and childhood body size, particularly in girls. Lower age of both B2 development and menarche has been consistently associated with increased body mass. Higher BMI values were seen in early maturers and lower average BMI in late maturers in both white and Afro-Caribbean girls. He et al. [10] demonstrated in a large data set (n = 3650) that one BMI unit increase between the ages of 2 and 8 years is associated with a 0.11 year advancement in the timing of puberty in both genders as measured by PHV. In contrast, undernutrition in females, for example, in chronic disease or anorexia nervosa, can result in delay in both the onset and tempo of puberty.

In boys the data are less consistent with some studies having noted an earlier onset of puberty with greater adiposity and some with a later onset. In particular, more European studies have noted the former trend, while US studies have more often shown the latter [11]. More recent data from the USA has shown a far more complex relationship between fat mass and pubertal timing, with overweight status being associated with earlier pubertal onset but obesity associated with later onset [12]. These effects also varied between ethnic groups. One hypothesis is that greater BMI in boys leads to earlier pubertal timing up to the threshold at which obesity occurs. Obesity may lead to later pubertal timing due to suppression of the HPG axis or via adiposity leading to excess aromatase activity and increased conversion of testosterone to oestrogen in boys.

The relationship between fat mass and pubertal timing is mediated, at least in part, through the permissive actions of the metabolic hormone leptin, a key regulator of body mass, produced from white adipose tissue (WAT) (Figure 7.1). Serum leptin concentrations increase in early female puberty and are required for normal reproduction. Humans and mice lacking leptin (Lep ob/ob) or the leptin receptor (LepR db/db) fail to complete puberty and are infertile. The action of leptin in influencing GnRH secretion is not clear-cut. In males, leptin concentrations decrease during puberty. Leptin does not act directly on GnRH neurons as they do not express the LepR. Leptin appears to regulate GnRH neurons indirectly by its action on the hypothalamus via cells that are afferent to GnRH neurons, such as LEPR-expressing gamma-aminobutyric acid (GABA) neurons from the arcuate nucleus (ARC), or via cells that interact morphologically with them, at least in part via the action of nitric oxide (which is required for its action) and via kisspeptin/neuropeptide Y (NPY) neurons [13]. These upstream regulators of GnRH secretion are discussed in more detail below ('Upstream control of GnRH neuronal function' section).

NPY is involved in many CNS functions, including appetite control and reproduction. NPY acts at the

hypophyseal level to potentiate LH secretion in response to GnRH by modulating GnRH binding to anterior pituitary GnRH receptors and at the level of the median eminence to stimulate GnRH secretion from GnRH axon terminals. NPY plays an important role in the metabolic control of fertility. Chronic increase in NPY tone inhibits LH and FSH, delays sexual maturation and suppresses oestrous cyclicity in rodents but acute changes in NPY may have variable effects depending on the levels of sex steroid production. Evidence from primate studies suggests that NPY may have a contributory role in the break restraining the onset of puberty in primates [14].

Ghrelin and other gut-derived peptides may also form part of the mechanism by which energy homeostasis regulates reproductive development. Ghrelin is the endogenous ligand for the GH secretagogue receptor and is produced primarily by gastric mucosa. Ghrelin circulates in the blood and stimulates the secretion of GH, prolactin and adrenocorticotropic hormone from the pituitary as well as hypothalamic control of food intake. Animal studies demonstrate that centrally or peripherally administered ghrelin reduces LH pulse frequency in ovariectomized rats and rhesus monkeys and decreases basal LH concentrations in intact rats and sheep. Both low birth weight and prematurity are associated with earlier onset of puberty, particularly in those children with rapid increase in length or weight in the first 2 years of life. It remains unclear, however, if childhood obesity, insulin resistance, excess androgens or other factors may explain this association.

Despite this body of evidence, additional data point to a downward trend in the age of puberty onset that is independent of BMI [15]. Moreover, while an ongoing strong secular trend towards earlier attainment of B2 has been recognized, the age of menarche in recent years, at least in Northern European studies, has not declined to the same extent. Indeed, as detailed above, some studies suggest that over the last decade the age of menarche and of completion of puberty in males in some populations has become skewed towards later ages. These data may imply that the increase in fat mass alone cannot explain this secular trend and suggest a role for factors that have an oestrogen-like effect, without central activation of the HPG axis.

The effect of possible endocrine-disrupting chemicals (EDCs) on the timing of puberty has been an ongoing concern [16]. Polybrominated biphenyls, bisphenol A, atrazine (herbicides) and phthalates among others have been suggested as possible EDCs responsible for contributing to this observed trend. For example, children migrating for international adoption and formerly exposed to the oestrogenic insecticide DTT in their country of origin displayed early or precocious pubertal timing. However, a clear mechanism of action for EDCs through the early initiation of the pulsatility of GnRH from the hypothalamus has not been conclusively demonstrated. Studies are complicated by the likely differing and possibly divergent influence of different doses and mixtures of EDCs and differing effects depending on age and length of exposure.

Epigenetic regulators are potential mediators of the effects of the environment on the hypothalamic regulation of puberty. However, while experimental data from rats give evidence for changes in histone acetylation and gene methylation leading to altered gene expression during puberty, the link between environmental factors and epigenetic control of puberty via the hypothalamus has not been established. While the window of opportunity for the effects of EDC exposure was historically considered to be in the late prepubertal period, evidence of fetal and neonatal origin of changes in pubertal timing counters this dogma. Prenatal exposure in boys to EDCs such as phthalates is associated with reduced masculinization of genital structures. Epigenetic changes during fetal life are a potential mechanism for the effects of EDCs *in utero*. The effects of EDCs may persist in pregnant rats not only in their unborn fetus but also into the next generation.

Genetic Regulation of Pubertal Timing

Despite the importance of environmental factors, genetic influence on the timing of puberty is clearly fundamental and, although the timing of pubertal onset varies within and between different populations, it is a highly heritable trait as shown by the high correlation of the timing of sexual maturation within families and in twin studies. Previous epidemiological studies and genetic approaches estimated that 50–80% of the variation in pubertal onset is under genetic control [17] but, despite this strong heritability, little is known about the control mechanisms. Attempts to identify key genetic regulators have ranged from genome-wide association studies (GWAS) of age at menarche examining pubertal timing in healthy women to next-generation sequencing (NGS) approaches to identify causal mutations in disease cohorts with delayed, absent or PP.

The existence of genetic heterogeneity is supported by several large GWAS. The first of many loci associated with age of menarche was the gene *LIN28B*, which is a human orthologue of the gene that controls developmental timing in the *Caenorhabditis elegans* through microRNAs. The lin-28 family regulates the biogenesis of let-7 microRNA (miRNA) family members controlling the timing of developmental events and in turn let-7 miRNA controls lin-28 translation. The major allele of the single nucleotide polymorphism (SNP) rs314276 (located in intron 2 of *LIN28B*) was associated with earlier age at menarche and earlier breast development in

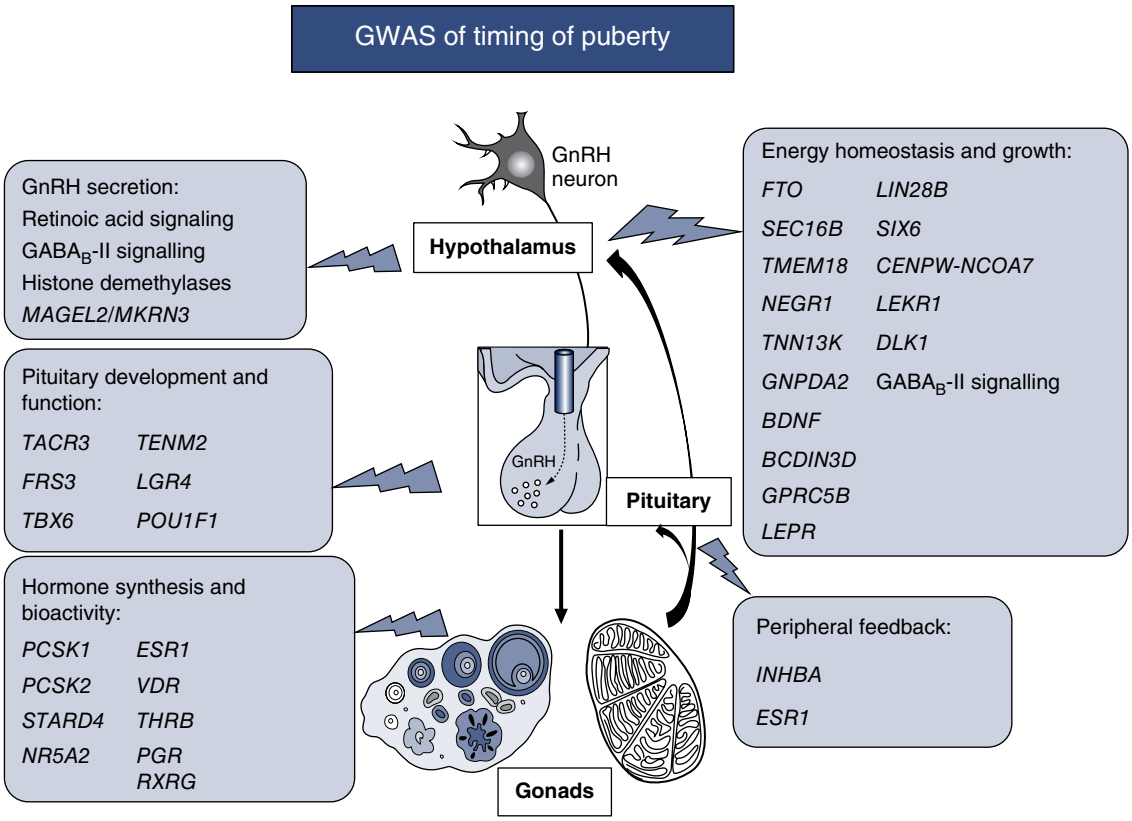

Figure 7.8 Possible roles in the hypothalamic–pituitary–ovarian axis of several of the implicated genes and biological mechanisms for menarche timing. *Source:* Adapted from Perry et al. [19], reproduced with permission of Springer Nature.

girls, earlier voice breaking and more advanced pubic hair development in boys and faster tempo of height growth and shorter adult height in both sexes.

In 2010, a large meta-analysis identified 42 (30 new, 2 previously confirmed and 10 possible) loci for age at menarche [18]. In 2014, this was extended to encompass data from genome-wide and custom-genotyping arrays in up to 182,416 women of European descent from 57 studies (Figure 7.8) [19]. Evidence ($P < 5 \times 10^{-8}$) for 123 signals at 106 genomic loci was identified. Many of these loci were associated with Tanner staging in both sexes, suggesting these data are applicable to both men and women. Further GWAS data appear to confirm this finding.

Importantly, for the first time, genes already identified in rare disorders of puberty were identified from a GWAS. These included the imprinted gene *MKRN3*, paternally inherited mutations that have been identified as causal in pedigrees of CPP. *MKRN3* is to date only the third gene with mutations identified as causal in pedigrees of central precocious puberty (CPP), the others being *KISS1* and its receptor *GPR54*.

Signals were also found near *LEPR–LEPROT*, which encodes the leptin receptor and is immediately upstream of *TACR3*, which encodes the neurokinin B receptor. A further variant ~10 kb from *GNRH1* approached genome-wide significance. Two signals were found near *PCSK1* and *PCSK2*, indicating a common function of the type 1 and 2 prohormone convertases in pubertal regulation. Finally, signals in or near several further genes with relevance to pituitary development and function including *POU1F1, TENM2* and *FRS3* and signals representing *cis-eQTLs* for *LGR4* and *TBX6,* which both encode enhancers for the pituitary development factor *SOX2*, were identified.

In addition to leptin signalling, the authors found overlap with several genes implicated in BMI including *FTO*, *SEC16B*, *TMEM18* and *NEGR1*. As discussed previously, puberty requires a minimum level of energy availability, while increased BMI has been shown to be associated with precocious onset of puberty. However, the molecular mechanisms for this are still unclear. Roles for other genes connected with regulation of body mass have not been clearly demonstrated in puberty. *FTO* (fat mass and obesity-associated gene) was the first obesity susceptibility gene identified through GWAS and continues to be the locus with the largest effect on BMI and obesity risk. *FTO* appears to exert this effect via influence on food intake regulation rather than physical exertion. A recent finding of the importance of *IRX3*, a second gene found

at the same GWAS locus, in influencing BMI briefly threw into doubt the primary role of *FTO*. However, the body of evidence behind *FTO*, in particular the results from *FTO* knockout mice and *in vitro* studies showing that FTO expression is regulated by essential amino acids and that it couples amino acid levels to mTORC1 signalling, has reinforced *FTO* as a major player in the regulation of body mass. A novel concept in the analysis of GWAS data is that a number of genes in any one identified region may play an important role in a particular phenotype. Whether such genes may regulate pubertal timing exclusively via impact on body mass or via other BMI-independent mechanisms is as yet unknown.

Pathway analyses implicated nuclear hormone receptors, particularly those involved in retinoic acid (RA) and GABA B2 receptor signalling. The active metabolites of vitamin A, all-trans-RA and 9-cis-RA, have differential effects on GnRH expression and secretion. Other possible mechanisms linking RA signalling to pubertal timing include inhibition of embryonic GnRH neuron migration and enhancement of steroidogenesis and gonadotropin secretion.

To date, nearly 400 loci have been shown to be associated with timing of menarche, explaining ~7.4% of the population variance, suggesting that many of these genetic variants have a low impact in the general population [20]. The authors hypothesize that the genetic architecture of the timing of puberty in healthy subjects involves hundreds of common variants. These studies rely on self-recall of the age at menarche, which may result in imprecise data. Research into the major genetic determinants of pubertal timing in the normal population is still in its infancy and only just beginning to generate feasible candidate genes.

Deletion mapping, homozygosity mapping in consanguineous kindreds with multiple affected members, targeted sequencing projects and, more recently, NGS approaches in patients with congenital hypogonadotropic hypogonadism (CHH) and familial PP have led to the identification of some of the key regulators of the HPG axis. Genetic mutations in over 30 separate genes resulting in severely delayed or absent puberty have now been described and several important gene discoveries have been made recently in patients with CPP. These genes include *ANOS1, FGFR1*, *FGF8*, *PROK2* and *PROK2R*, *CHD7* and *NSMF*, *GNRH1* and *GNRHR*, *KISS1* and *KISS1R*, *TAC3* and *TACR3, SEMA3A, SOX10, IL17RD, FEZF1, WDR11, AXL, HS6ST1* and *FGF17* in hypogonadotropic hypogonadism (HH) and *MKRN3, KISS1R* and *DLK1* in CPP. These genes are involved in the control of GnRH neuronal migration and differentiation, GnRH secretion or its upstream or downstream pathways.

Although these discoveries are critical in enhancing the understanding of the regulation of the hormonal pathways, ~50% of the genetic causes of HH are still unknown. There is a greater or lesser degree of phenotypic variability depending on the gene involved, with nearly all mutations in *ANOS1* leading to HH with anosmia (KS) whereas loss-of-function mutations in *FGFR1* have been identified in pedigrees with KS, normosmic HH and DP. Environmental factors may go some way to explaining these variations but it is increasingly clear that gene–gene interactions in HH are an important phenomenon and strategies to identify such digenic and even oligogenic inheritance in families are developing. In such kindreds, the pattern of those affected by disease may not conform to classic Mendelian inheritance dogma and bioinformatic filtering pipelines and statistical modelling techniques will require modification to identify novel candidates for such gene–gene interactions.

The Hypothalamic–Pituitary–Gonadal Axis

Development of the GnRH Neuronal Network

The development of the HPG axis is exceptional in that GnRH neurons develop in metazoan embryos outside the central nervous system (CNS). Immature GnRH precursor neurons are first detectable in the olfactory placode in the nose from an early embryological stage (E10.5–E11 in mice) and then begin a complex journey towards the hypothalamus.

The embryonic migration of GnRH neurons from the nose to hypothalamus is key for the development of the neuroendocrine pathways that allow normal pubertal development [21]. Despite great progress in the understanding of this migratory process, the origin of GnRH neuronal precursors remains unclear. Hypotheses as to their derivation have included nasal placode cells, adenohypophyseal precursors or neural crest-derived cells. In mammals, GnRH1 expression can be detected preimplantation in the blastocyst and morula stages but is first detected in the developing head within the nasal placode. GnRH neurons develop in the nasal placode as a cell population distinct from the developing olfactory epithelium. While GnRH cells share some markers in common with neural crest cells, evidence for a direct lineage had been difficult to establish but data from Cre-lox lineage tracing has led to the current consensus that GnRH neurons are of mixed lineage, with around 30% being neural crest derived and the remainder being of placodal ectoderm origin.

The pathway along which the GnRH neurons travel is long and within a scaffold formed by olfactory, vomeronasal and terminal nerves 3 and 4 at different stages. Cells begin their migration in the nasal compartment in or around the vomeronasal organ, cross the cribriform plate and penetrate the brain in close proximity to the

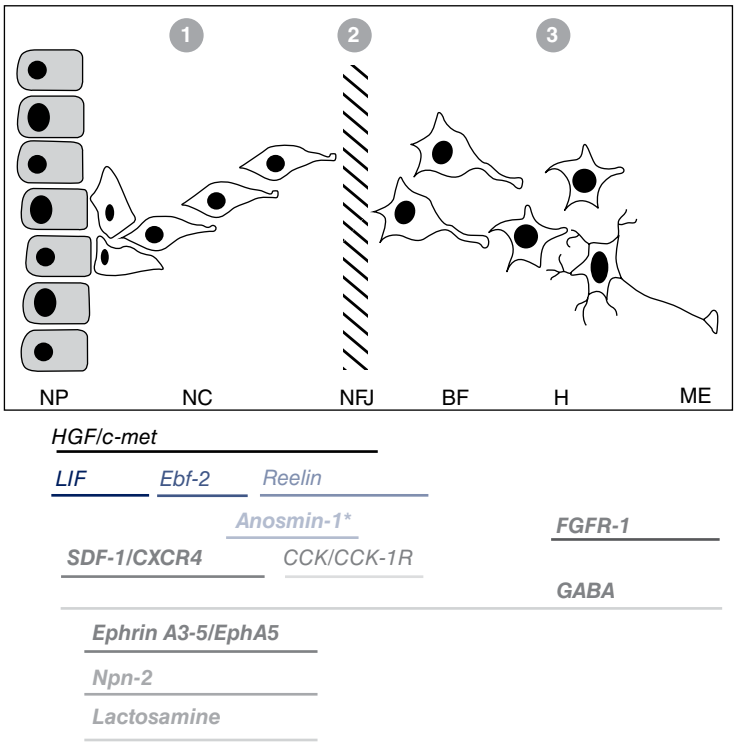

Figure 7.9 Factors that affect the migration of gonadotropin-releasing hormone (GnRH) neurons through the three compartments. The illustration shows the movement of GnRH neurons from their origin in the nasal placode (NP) through the nasal compartment (NC) and their deflection at the level of the nasal–forebrain junction (NFJ) as they progress towards the basal forebrain (BF). Their migration terminates in the hypothalamus (H) from where they project to the median eminence (ME). Factors that have been shown to affect GnRH neurons at different stages of their journey are shown below. HGF, hepatocyte growth factor; LIF, leukaemia inhibitory factor; Ebf-2, early B-cell factor 2; SDF1, stromal cell-derived factor 1; CXCR4, CXC chemokine receptor type 4; CCK, cholecystokinin; Npn-2, neoplastic progression 2. *Source:* From Cariboni et al. [21], reproduced with permission of Elsevier.

olfactory bulbs and finally migrate apposed to a subset of vomeronasal nerves that diverge caudally into the basal forebrain. At the end of their journey, GnRH neurons dissociate from guiding axons to disperse into their final positions of the septo-hypothalamic region, including the medial septum, the diagonal of Broca and the preoptic area of the hypothalamus. GnRH neurons extend their neurites to the median eminence under the control of mostly unknown factors. FGFR1 signalling has been shown to be important for this process of axon extension, with reduced projections to the median eminence in transgenic mice expressing a dominant negative FGF receptor (dnFGFR) in GNRH1 neurons.

GnRH neurons are known to have receptors for at least 20 neurotransmitters. Migratory GnRH neurons receive a plethora of guidance and movement-inducing messages during this journey, which are likely to be distinct depending on the stage of their migration (Figure 7.9) [21]. Signals may act directly or indirectly through the extension of olfactory axons, as disruption of the nerve tract 'scaffolds' themselves can disrupt GnRH migration. The molecular signals involved include those controlling cell–cell interactions (membrane receptors [e.g. neuropilin-2], adhesion molecules [e.g. NCAM], extracellular matrix molecules [e.g. heparin sulphotransferases], cytokines [e.g. LIF, HGF], and transcription factors [e.g. Ebf-2]) as well as both chemoattractants and chemorepellents (e.g. Reelin). Gradients of chemokines (e.g. SDF1, also known as CXCL12) may be particularly important for promoting movement of GnRH neurons. This combination of factors has a high degree of redundancy, which is necessary given the crucial role this GnRH neural network plays in reproductive function.

ANOS1, previously known as *KAL1*, encodes anosmin-1, an extracellular matrix protein that regulates axonal path finding and cellular adhesion. Anosmin-1 promotes branching of olfactory bulb neurons. Subjects with *ANOS1* loss-of-function mutations have arrest of both GnRH neurons and olfactory bulb neurons at the cribriform plate. It is not yet clear whether the effects of anosmin-1 are limited to the development of olfactory neurons or whether it has an additional chemotactic influence on GnRH neurons. Although no mouse model is available, fish and nematode studies and *in vitro* work have further elucidated the role of *ANOS1*.

NMDA Receptor Synaptonuclear Signaling And Neuronal Migration Factor (NSMF) also appears to have an important role as a common guidance molecule for olfactory axon projections and subsequently either directly or indirectly in the migration of GnRH neurons. Perturbations of this migratory journey have been repeatedly shown to lead to HH in humans and in animal models [22] (Figure 7.10).

The whole process of migration involves no more than a few hundred neurons per hemisphere in mouse (several thousands in primate or human). The absolute number of GnRH neurons required for pubertal development is not known but there appears to be a degree of redundancy in the system. Rodent studies suggest that around

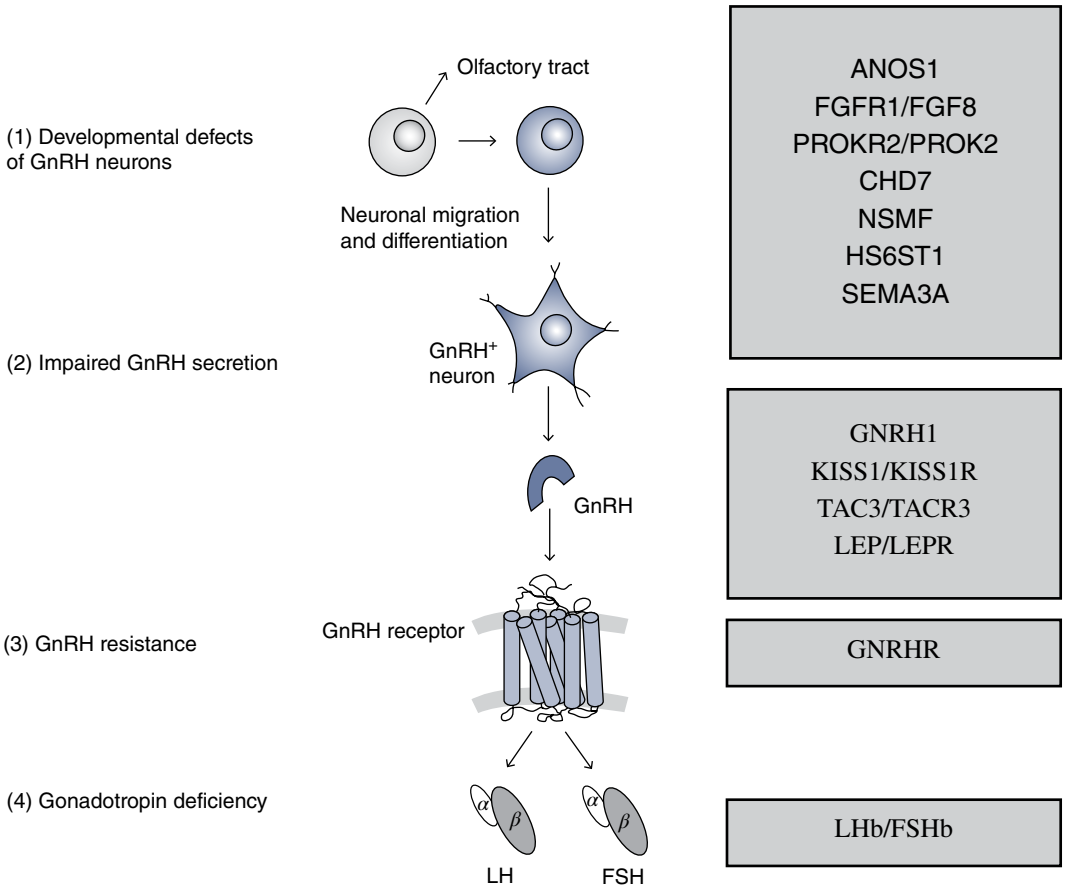

Figure 7.10 Mutations in single genes at many levels of the HPG axis can cause hypogonadotropic hypogonadism. *Source:* Adapted from Beate et al. [22]. Licensed under CCBY 4.0.

12% of the GnRH neuron population is sufficient for pulsatile gonadotropin secretion and puberty onset, whereas between 12 and 34% are required for cyclical control in adult female mice. In addition, adult reeler mice have significantly fewer GnRH neurons in the hypothalamus and display a phenotype of delayed pubertal maturation and low fertility.

This full developmental sequence, completed by the eighth to ninth week of embryonic development, can therefore be compartmentalized into several discrete but well-coordinated events, starting with (1) fate specification of GnRH neurons, (2) expansion of cell numbers (mitosis and apoptosis), (3) cell migration (a mixture of chemo-repulsive and chemo-repellent events), (4) coalescence of individual GnRH neurons into a responsive, secreting and coordinating network functioning in an integrated manner and (5) the development of a capacity to incorporate and integrate internal and external feedback signals into the final feedback control mechanisms that modulate GnRH release. Mutations in the genes whose actions determine any one or more of these pathways could theoretically underpin congenital forms of HH. A further tier of regulation of these neurosecretory events involves the reversible detachment of these nerve endings onto the capillary loops of the median eminence postnatally.

Thus the genes whose loss of function results in HH can broadly be classified into four groups (Figure 7.10):

1) Genes that appear to represent purely neurodevelopmental genes whose loss of function affects the development and migration of GnRH neurons into the hypothalamus (*ANOS1*, *NSMF*, *fibroblast growth factor receptor 1* [*FGFR1*], *fibroblast growth factor 8* [*FGF8*] and its synexpression group, *PROKR2*, *PROK2*, *CHD7*, *SEMA3A*, *SEMA3E*, *HS6ST1*, *WDR11*).
2) Genes that appear to be involved in GnRH synthesis and release, 'Upstream control of GnRH neuronal function' section (*GnRH1*, *KISS1*, *KISS1R*, *TAC3*, *TACR3*, leptin and its receptor, *SF1*, *DAX1*)
3) Genes involved in GnRH action, 'GnRH resistance and gonadotropin deficiency' section (*GnRHR*).
4) Genes involved in gonadotropin synthesis, 'GnRH resistance and gonadotropin deficiency' (*LHβ, FSHβ*).

GnRH is a 10-amino acid peptide generated from a large 69-amino acid prohormone precursor. The gene encoding GnRH is located on chromosome 8. Although GnRH is mainly located in the hypothalamus in adults, it is also found in the hippocampus, cingulate cortex and olfactory bulbs. There is no discrete nucleus that contains all of the GnRH neurons. GnRH is secreted in a pulsatile fashion into the hypothalamic–pituitary portal system by nerve terminals located in the median eminence to reach the anterior pituitary where it stimulates the secretion of the gonadotropins LH and FSH by pituitary gonadotroph cells.

GnRH is released in episodic boluses with a half-life of only 2–4 minutes and daily metabolic clearance of 800 L/m^2. The secretion of GnRH pulses is synchronized between GnRH neurons, such that they integrate their firing rates to generate an appropriate burst of GnRH release into the portal system. This synchrony is a complex process involving spontaneous electrical activity of the neurons, calcium and cAMP signalling, autocrine regulation through the GnRH receptor and regulation through other cell membrane receptors on these neurons.

The master controller of this GnRH neuronal pulse generator has been a subject of much research over recent years. The theory that the pulse generator is an intrinsic property of GnRH neurons has been mostly rejected, given evidence derived from models such as retrochiasmatic rat hypothalamic explants, which contain few if any GnRH cell bodies but continue to exhibit pulsatile GnRH release in culture. The 'KNDy' model of pulse generation, where key neurons in the ARC are responsible for coordinating pulse generation through the peptides, kisspeptin, neurokinin B and dynorphin, is discussed in more detail in the next section [14].

Upstream Control of GnRH Neuronal Function

GnRH release is coordinated through a balance of inhibitory and excitatory neuronal and glial inputs [23] (Figure 7.11). Retrograde tracing studies in mice have shown that GnRH neurons are subject to a complex neuronal network of inputs from many regions of the brain including hypothalamic nuclei, the brainstem, limbic system, basal ganglia and motor and sensory circuits.

Among various regulators of GnRH neurons, kisspeptins and neurokinin B are essential. Kisspeptin, an excitatory neuropeptide, was identified as a vital permissive factor in puberty onset by the discovery of patients with GnRH deficiency with loss-of-function mutations in the *KISS1* receptor, *KISS1R*, previously known as *GPR54* [24, 25]. Mice with knockout of *Kiss1r* were simultaneously discovered to be infertile despite anatomically normal GnRH neurons and normal hypothalamic GnRH levels [25]. Their phenotype can be rescued by exogenous delivery of GnRH. *Kiss1* knockout mice also have a phenotype consistent with normosmic GnRH deficiency.

Kisspeptins are synthesized by hypothalamic neurons that are in close contact with GnRH neurons. Most GnRH neurons express the kisspeptin receptor and kisspeptin neurons express steroid receptors including the oestrogen receptor alpha, the progesterone receptor and the androgen receptor (AR). These neurons are the main relay for the negative and positive feedback of steroid hormones on the gonadotropic axis. Axonal ends of kisspeptin neurons project not only to the GnRH neuron cell body in the OVLT but also to the median eminence where they are in apposition with the extremities of GnRH neurons. Kisspeptin neurons are located outside the blood–brain barrier and therefore directly in contact with peripheral hormones.

Kisspeptin thus signals directly to GnRH neurons to control pulsatile GnRH release. It is upregulated in both primates and mice in the peri-pubertal period and its administration in prepubertal rodents advances the onset of puberty. Kisspeptin also appears to be downregulated in functional amenorrhoea, suggesting its role as a mediator of the action of environmental factors such as nutritional status and emotional well-being on puberty and reproductive capacity. Additionally, kisspeptin has been shown to be an important neuroendocrine regulator of ovulation.

The distribution of kisspeptin neurons in the hypothalamus is complex and varies with species. In mice, Kiss1 neurons reside within the hypothalamus caudally, in the mediobasal hypothalamus, which includes the ARC/infundibular region, but a second population is located in the rostral part of the hypothalamus called the anteroventral periventricular (AVPV) region. In rats, monkeys and humans, this regional distribution of kisspeptin is less clear.

The two populations of kisspeptin neurons in mice exhibit marked functional differences. AVPV Kiss1 neurons exhibit clear sexual dimorphism, with female rodents at puberty having a much greater number of Kiss1 neurons in this area. Kisspeptin neurons located in the AVPV mediate the positive feedback of oestradiol on the GnRH neuronal network and therefore the generation of the LH preovulatory pulse. In contrast, peripheral oestrogens have a primarily inhibitory effect on Kiss1 neurons in the ARC. Thus, kisspeptin signalling is an important element of both positive and negative feedback loops in the HPG axis. While kisspeptin has been identified as a pivotal upstream regulator of GnRH neurons, whether it is the key factor in triggering the onset of puberty remains unclear [26].

An additional excitatory neuropeptide, neurokinin B, has been implicated in the upstream control of GnRH secretion. Identification of this pathway was also via

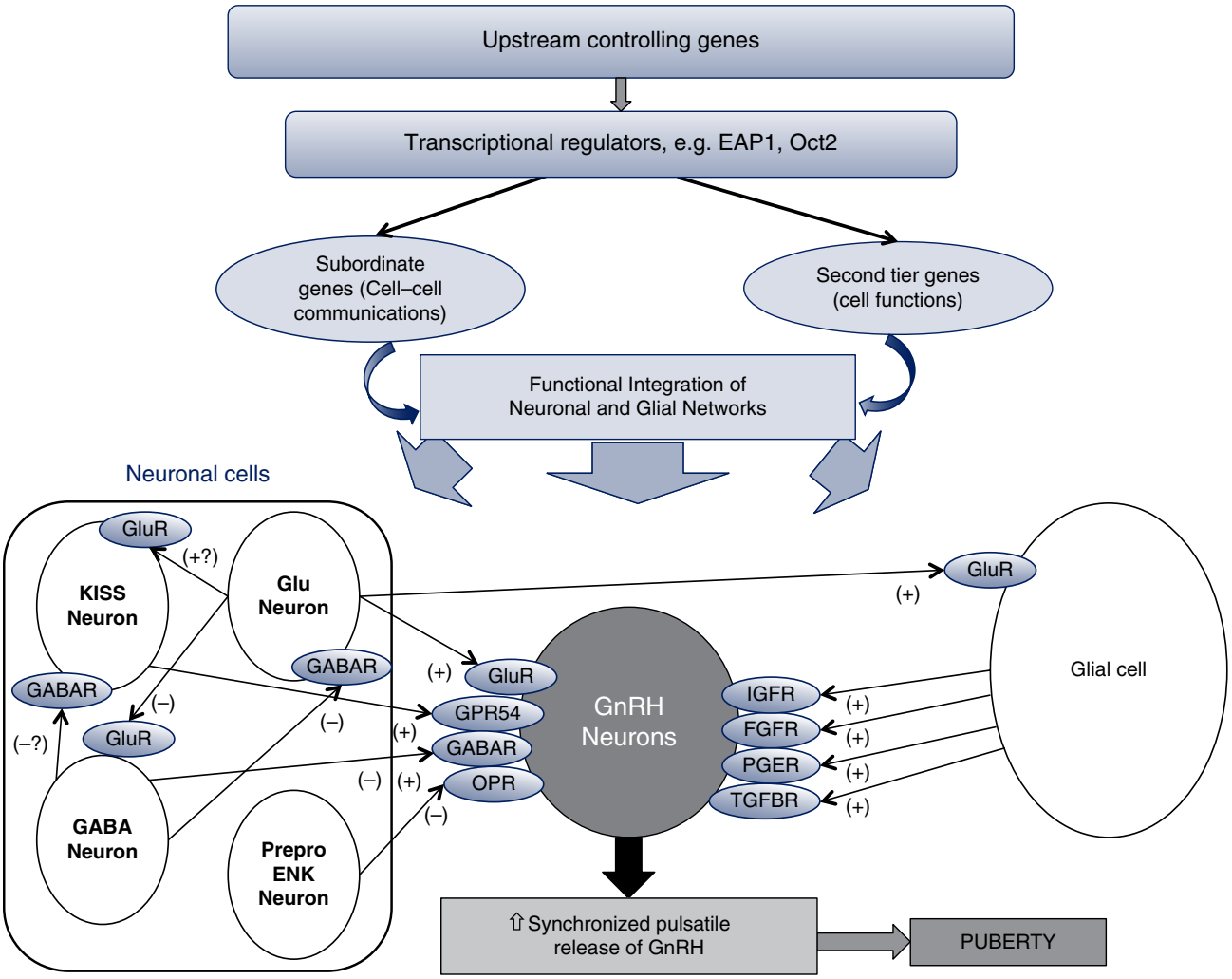

Figure 7.11 Genetic regulators in the trans-synaptic and glial control of GnRH neurons during puberty. *Source:* Adapted from Ojeda et al. [23], reproduced with permission of Oxford University Press.

discovery of loss-of-function mutations in *TAC3*, encoding neurokinin B, and its receptor *TACR3* in patients with normosmic HH and pubertal failure [27]. Kisspeptin neurons located in the ARC synthesize neurokinin B and dynorphin A; they form KNDy neurons. Both *KISS1* and *TAC3* expressions in the ARC are downregulated by oestrogen and these neurons are considered as the relay of the negative feedback of steroid hormones on the gonadotropic axis. KNDy neurons express the neurokinin receptor, NK3R, suggesting that autocrine and paracrine loops control GnRH release. Dynorphin inhibits the release of GnRH and together these peptides are believed to play a fundamental role in the GnRH pulse generator.

Another RF-amide-related peptide (RFRP1, RFRP3), the mammalian orthologue of the avian peptide *gonadotropin-inhibiting hormone* (GnIH), has emerged as a further inhibitory regulator of the gonadotropic axis by directly controlling GnRH neurons. GnIH plays a crucial role in the inhibitory regulation of the HPG axis in several species. In addition to neuropeptides, several neurotransmitters participate in the control of the GnRH network. In the ARC, GABA and glutamate control GnRH neuronal excitability. In female rats, glutamine synthase is downregulated and glutamate dehydrogenase becomes more abundant in the hypothalamus at puberty, both leading to increased availability of glutamate. Glutamate antagonists are potent stimulators of GnRH secretion and administration to prepubertal primates can stimulate the onset of puberty. The GABA neural network is quite complex since some of these neurons will have a direct effect on GnRH neurons and others will act on interneurons. The inhibitory role of GABAergic neurotransmission in restraining the initiation of puberty has been clearly shown in primates but is more ambiguous in rodents. GABAergic signalling pathways are likely to be important in the stress-induced suppression of LH.

Recent evidence highlights the importance in mice of microRNAs (particularly the miR-200/429 family and miR-155) in the epigenetic upregulation of GnRH transcription during the critical period (murine equivalent of the mini-puberty) [28]. Moreover, miR-7a2 has been demonstrated to be essential for normal pituitary development and HPG function, with deletion in mice leading to hypogonadotropic infertility.

Central corticotropin-releasing hormone (CRH) signalling has long been known to play an important role in the stress-induced suppression of the GnRH pulse generator in rodents. Juxtaposition of CRH immunoreactive fibres to GnRH neurons has been observed in the human hypothalamus in the infundibular region. Intracerebroventricular (ICV) administration of CRH decreases LH pulse frequency in rats whereas stress-induced suppression of LH pulses by insulin-induced hypoglycaemia is blocked by ICV administration of a CRH antagonist.

The synchronous pulsatile secretion of GnRH is also controlled through the activation of neuron/glia signalling pathways. Glial inputs appear to be predominantly facilitatory and consist of growth factors and small diffusible molecules, including TGFβ1, IGF-1 and neuregulins, that directly or indirectly stimulate GnRH secretion [29]. Firstly, glial cells in the median eminence regulate GnRH secretion by production of growth factors acting via receptors with tyrosine kinase activity. FGF signalling is required for GnRH neurons to reach their final destination in the hypothalamus, as well as for GnRH neuronal differentiation and survival. Additionally, GnRH neuron secretory activity is facilitated by IGF-1 and by members of the epidermal growth factor family such as neuregulin 1β. Secondly, plastic rearrangements of glia-GnRH neuron adhesiveness mediated by soluble molecules such as neuronal cell adhesion molecule (NCAM) and synaptic cell adhesion molecule (SynCAM) coordinate the controlled delivery of GnRH to the portal vasculature, a process that is also subject to sex steroid regulation.

Downstream Pathways of GnRH Action

Gonadotropins

GnRH stimulates the production and secretion of LH and FSH from the gonadotrophs by binding to a cell surface receptor that triggers increased intracellular calcium concentration and phosphorylation of protein kinase C in a manner similar to other peptide–receptor mechanisms. There appear to be readily releasable pools of LH that lead to an increase in serum LH within minutes after a bolus of GnRH as well as other pools of LH that take longer to mobilize. While episodic stimulation by GnRH increases gonadotropin secretion, continuous infusion of GnRH decreases LH and FSH secretion and downregulates the pituitary receptors for GnRH. This phenomenon is used in the treatment of central precocious puberty (CPP). Oestrogens increase and androgens decrease GnRH receptors. These alterations in the GnRH receptor have an important role in regulating gonadotroph function.

FSH and LH are glycoproteins composed of two subunits, an α-subunit that is identical for all the pituitary glycoproteins and distinct β-subunits that confer specificity. The β-subunits are 115 amino acids long with two carbohydrate side chains. Human chorionic gonadotropin (hCG) produced by the placenta is almost identical in structure to LH except for an additional 32 amino acids and additional carbohydrate groups. The LH β-subunit gene is on chromosome 19q13.32, close to the gene for β-hCG, while the FSH β-subunit gene is located at 11p13. There are rare cases of mutations in the β-subunit of gonadotropin molecules that cause pathological effects: a single case of an inactivating mutation of β LH caused the absence of Leydig cells and lack of puberty while two cases of inactivating mutations of β FSH led to the lack of follicular maturation and amenorrhoea and, in two males, azoospermia. Additionally, a woman with a homozygous mutation in a 5′ splice donor site in the non-coding region of β LH displayed impaired LH secretion, normal pubertal development, secondary amenorrhoea and infertility [30].

The mechanism for this is unclear. LH secretion could not be detected by two separate immunoassays suggesting that the mutant LHB protein might be translated but unable to associate with the α-subunit or was translated but rapidly degraded or was not translated at all. What is important from these observations is that normal pubertal maturation in women, including breast development and menarche (which indicate oestrogen production sufficient for breast development and at least some tropic action on the endometrium), can occur in a state of LH deficiency, although normal LH secretion is obligatory for ovulation. This implies that LH is essential for the normal maturation of Leydig cells and steroidogenesis in men and that its primary role in women is to induce ovulation.

The same gonadotroph cell produces both LH and FSH. The gonadotrophs are distributed throughout the anterior pituitary gland and abut upon the capillary basement membranes to allow access to the systemic circulation. Inactive gonadotroph cells that are not stimulated, e.g. due to disease affecting GnRH secretion, are small in diameter, while the gonadotroph cells of castrate individuals or those with the absence of gonads such as in Turner syndrome, which are stimulated by large amounts of GnRH, are large in diameter and demonstrate prominent rough endoplasmic reticulum.

Serum gonadotropin concentrations change during the progression of pubertal development (Table 7.2). Because of the episodic nature of gonadotropin secretion, a single gonadotropin determination will not reveal information about the secretory dynamics of these hormones.

Table 7.2 Serum gonadotrophins, gonadal and adrenal steroids in stages of pubertal development in (a) girls and (b) boys.

(a) Girls				
Tanner stage	**LH (IU/L)**	**FSH (IU/L)**	**Oestradiol (pg/mL)**	**DHEAS (μg/dL)**
1	0.01–0.21	0.50–2.41	5–10	5–125
2	0.27–4.21	1.73–4.68	5–115	15–150
3	0.17–4.12	2.53–7.04	5–180	20–535
4	0.72–15.01	1.26–7.37	25–345	35–485
5	0.30–29.38	1.02–9.24	25–410	25–530
(b) Boys				
Tanner stage	**LH (IU/L)**	**FSH (IU/L)**	**Testosterone (μg/dL)**	**DHEAS (μg/dL)**
1	0.02–0.42	0.22–1.92	2–23	5–265
2	0.26–4.84	0.72–4.60	5–70	13–380
3	0.64–3.74	1.24–10.37	15–280	60–505
4	0.55–7.15	1.70–10.35	105–545	65–560
5	1.54–7.00	1.54–7.00	265–800	165–500

Values are taken from standards of Quest Diagnostics with permission.
DHEAS, dehydroepiandrosterone sulphate; FSH, follicle-stimulating hormone; LH, luteinizing hormone.

However, newer third-generation assays are sufficiently sensitive to indicate the onset of puberty in single basal unstimulated samples, but the role of this form of clinical assessment remains unclear.

GnRH must stimulate gonadotropin release before any other factors can affect gonadotropin secretion but, in the presence of GnRH stimulation, sex steroids and gonadal peptides can change gonadotropin secretion. Negative feedback inhibition is manifest when sex steroids decrease pituitary LH and FSH secretion at the hypothalamic and pituitary levels and is exemplified in individuals with gonadal dysgenesis who have very high concentrations of LH and FSH during infancy and puberty. The protein inhibin, a product of both ovary and testes, and follistatin, an ovarian product, also exert direct inhibitory effects upon FSH secretion at the pituitary level. Progesterone slows the LH pulse frequency.

Gonadal Hormone Production

Sex Steroids The Leydig cells of the testes synthesize testosterone through a series of enzymatic conversions for which cholesterol is the precursor. When LH binds to Leydig cell membrane receptors, the ligand–receptor complex stimulates membrane-bound adenyl cyclase to increase cyclic adenosine monophosphate (cAMP), which then stimulates protein kinase, which in turn causes the stimulation of the conversion of cholesterol to pregnenolone by P450scc (side-chain cleavage enzyme), the first step in the production of testosterone. After exposure to LH, the number of receptors for LH and the post-receptor pathway decreases their responsiveness to LH for at least 24 hours. This explains the clinical finding of insensitivity to LH after daily injections of LH or hCG compared with every-other-day injections. When assessing the response of testes to LH, hCG or LH must be administered at 2–3 day intervals to eliminate such downregulation.

When testosterone is secreted into the circulation, the majority is bound to sex hormone-binding globulin (SHBG). The remaining free testosterone (95% of which is bound to albumin with low affinity) is conventionally considered the active moiety. At the target cell, testosterone dissociates from the binding protein, diffuses into the cell and may be converted by 5α-reductase type 2 (a surface enzyme located on the genital skin and elsewhere and encoded by a gene on chromosome 2) to dihydrotestosterone or by aromatase (CYP19) to oestrogen. Testosterone or dihydrotestosterone binds to the AR that is encoded by a gene on the X chromosome (Xq11–q12). The testosterone/dihydrotestosterone receptor complex then attaches to the steroid-responsive region of genomic DNA to initiate androgen-dependent transcription and translation.

The effects of testosterone are different from those of dihydrotestosterone since a fetus without dihydrotestosterone will not virilize fully. The AR has a greater affinity for dihydrotestosterone than for testosterone. Testosterone will suppress LH secretion, maintain Wolffian ducts and produce the male body habitus while dihydrotestosterone is mostly responsible for the virilization of the external genitalia and for much of the

secondary sexual characteristics of puberty including phallic growth, prostate enlargement, androgen-induced hair loss and beard growth. Androgens exert other effects in the body: testosterone promotes muscle development, stimulates enzymatic activity in the liver and stimulates haemoglobin synthesis. Androgens must be converted to oestrogen to stimulate bone maturation at the epiphyseal plate.

FSH binds to specific receptors on the cell surface of Sertoli cells and causes a sequence of events that culminates in increased protein kinase in a manner similar to the stimulatory effect of LH on Leydig cells. However, FSH causes an increase in the mass of seminiferous tubules and in an undefined way supports the development of sperm.

Oestrogen is produced mainly by the granulosa cells of the ovary with the same initial steps as testosterone production with a final aromatization process. In the female, LH binds to membrane receptors of ovarian cells and stimulates the activity of adenyl cyclase to produce cAMP, which stimulates the production of the low-density lipoprotein (LDL) receptor to increase binding and uptake of LDL cholesterol and the formation of cholesterol esters. LH stimulates the rate-limiting enzyme P450scc, which converts cholesterol to pregnenolone, initiating steroidogenesis. After the onset of ovulation, LH exerts major effects upon the theca of the ovary. FSH binds to its own cell surface receptors on the granulosa cells and stimulates the conversion of testosterone to oestrogen.

The main active oestrogen in humans is oestradiol. Oestrogens circulate bound to SHBG and follow the same general pattern of action at the cell level as described for testosterone. Oestradiol affects breast and uterine development, the distribution of adipose tissue and bone mineral accretion. Low concentrations are difficult to measure in standard assays. Oestradiol decreases gonadotropin secretion at low concentrations but causes positive feedback at higher values. This developmental stage predominates in females at approximately mid-puberty. By this process, a rising concentration of oestradiol >200–300 pg/mL persisting for more than 48 hours triggers the release of a burst of LH from the pituitary gonadotrophs, which stimulates ovulation about 12 hours later.

Several steps must prepare the HPG axis for positive feedback, including an adequate pool of LH for release, and priming of the ovary to produce adequate oestrogen; these events are kisspeptin dependent. Oestradiol also increases pituitary gland sensitivity to GnRH, which, in addition to an increase in GnRH pulse frequency, increases LH secretion. Thus, a follicle must be of adequate size to produce oestrogen adequate to exert the positive feedback effect, the pituitary gland must have sufficient readily releasable LH to effect a surge of LH release and the hypothalamus must be able to secrete adequate GnRH to cause the stimulation of pituitary release. The increase in oestrogen also suppresses FSH to allow luteinization of the follicle in the presence of LH.

Activin and Inhibin Inhibin B is a heterodimeric glycoprotein member of the transforming growth factor β family produced in males exclusively by the testis, primarily in the prepubertal testis by the Sertoli cells, and by the ovarian granulosa cells and the placenta in females. Several studies show that serum inhibin B concentrations in children change in concert with the secretion of gonadotropins [31]. During the 'mini-puberty', serum inhibin B concentrations increase to similar or higher concentrations to those observed in adolescent boys and adult men. This early inhibin B secretion is sustained until the age of 18–24 months; thereafter, serum concentrations decline to lower but readily measurable concentrations. Early in puberty, between Tanner stages G1 and G2, serum inhibin B concentrations again increase and reach peak concentrations at Tanner stage G2 but then plateau. Inhibin suppresses FSH secretion from the pituitary gland and provides another explanation for different serum concentrations of LH and FSH with only one hypothalamic peptide (GnRH) stimulating them.

Activin is a subunit of inhibin and has the opposite effect, stimulating the secretion of FSH from the pituitary gland. Absence of inhibin due to gonadal failure causes a greater increase in serum FSH than LH in pubertal and adult subjects.

Anti-Müllerian Hormone Anti-Müllerian hormone (AMH) belongs to the same transforming growth factor-β family as inhibin and is produced from the Sertoli cells of the testes from the time of testicular differentiation to puberty and in females by the granulosa cells from birth until menopause [32, 33]. In normal males, AMH is high in the fetus and newborn with peak concentrations around 2 months of age and then decreases by the age of 1 year. Patients with dysgenetic testes have decreased serum AMH while values are elevated in males with Sertoli cell tumours or females with granulosa cell tumours. Undetectable AMH and inhibin B are characteristic of congenital anorchia but may also be seen in males with severe IHH. A similar pattern in AMH concentrations during the first months of life has also been reported in infant girls, but the concentrations in girls are significantly lower. AMH decreases during puberty as a sign of androgen action. In girls, concentrations are considered a novel marker for follicular reserve. This has importance, for example, in Turner syndrome for assessment of potential reproductive capacity.

The Reactivation of the Gonadotropic Axis at Puberty

The gonadotropic axis undergoes a complex cycle of activation–inhibition from fetal life to puberty (Figure 7.12). Fetal testosterone secretion early in pregnancy is caused by placental hCG stimulation. The fetal hypothalamus contains GnRH-containing neurons by 14 weeks of gestation and the fetal pituitary gland contains LH and FSH by 20 weeks. The hypothalamic–pituitary portal system develops by 20 weeks of gestation, allowing hypothalamic GnRH to reach the pituitary gonadotrophs. At mid-gestation, there is a striking increase of circulating gonadotropin concentrations in both male and female fetuses that reaches its peak at 34–38 weeks in the male fetus and then falls to low concentrations at birth [35]. This change in gonadotropin secretion results from the development of a negative feedback system through sex steroids, as well as from the development of inhibiting influences from the CNS on GnRH neurons [36, 37].

LH and FSH secretion increases during the first month after birth, probably because the negative feedback effect of placental oestrogens is withdrawn. LH is secreted in pulses during this postnatal period, termed the 'mini-puberty'. After this postnatal activity, the HPG axis becomes dormant between the ages of 2 and 8–9 years. The suppression is not absolute as LH pulsatility is detectable during this stage using ultrasensitive assays, but pulses are infrequent and of low amplitude. Even in children without gonadal function, such as those with Turner syndrome, serum gonadotropin concentrations are low, demonstrating that the presence of the gonads is not necessary to suppress gonadotropin secretion during this period. Changes in gonadotropin secretion arise as a result of alterations in pulse amplitude with pulse frequency unchanged. Likewise, testosterone and oestrogen are measurable in the circulation using sensitive assays, demonstrating low but definite activity of the prepubertal gonads.

The first biological change demonstrating that the HPG axis is being reactivated at puberty is the augmentation of nocturnal LH pulses in children before clinical development of Tanner genital or breast stage 2. This period may thus be seen as the hormonal onset of

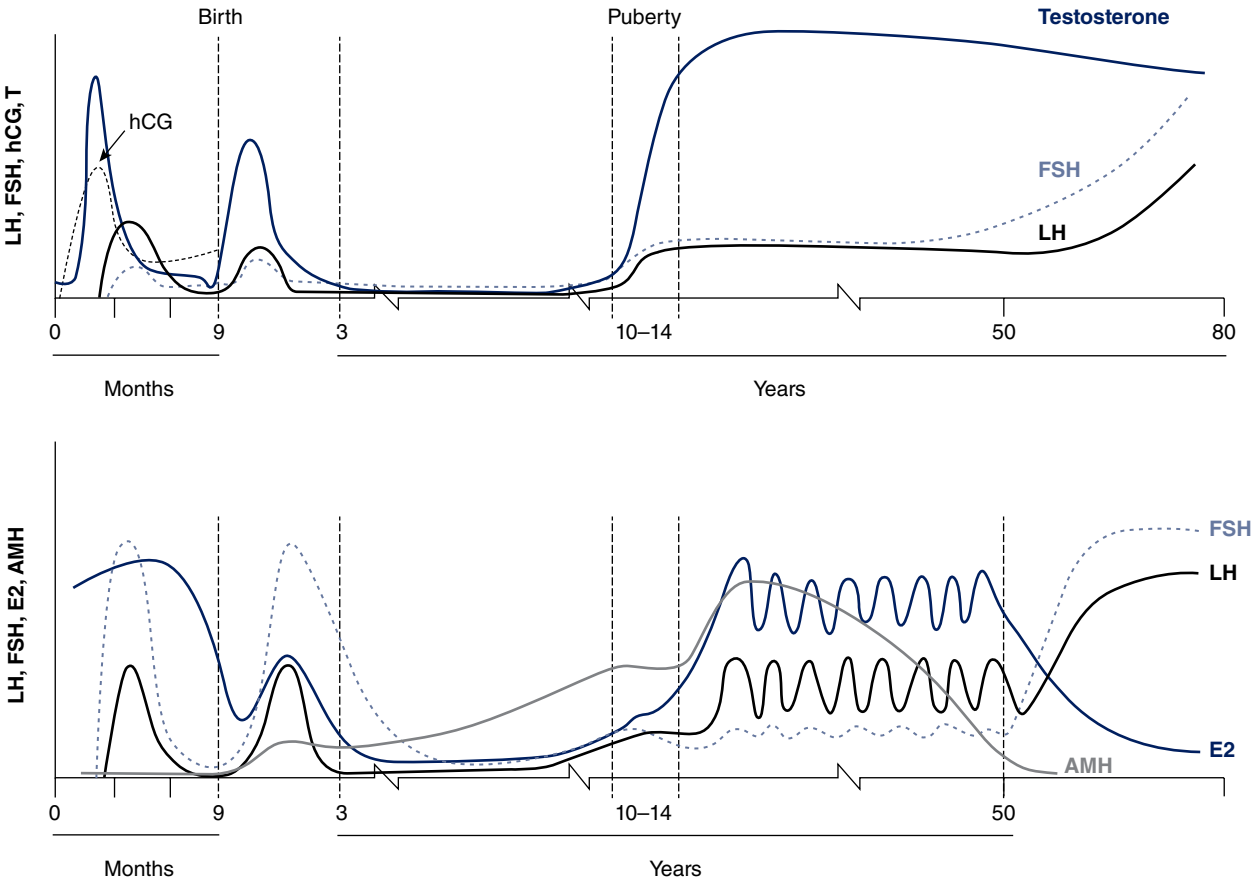

Figure 7.12 The HPG axis during fetal and postnatal life. Circulating concentrations of gonadotropins (hCG, FSH, LH) and testosterone during the lifespan of a male (top panel) and gonadotropins, oestradiol and anti-Müllerian hormone during the lifespan of a female (lower panel). *Source:* From Huhtaniemi et al. [34], reproduced with permission of Elsevier.

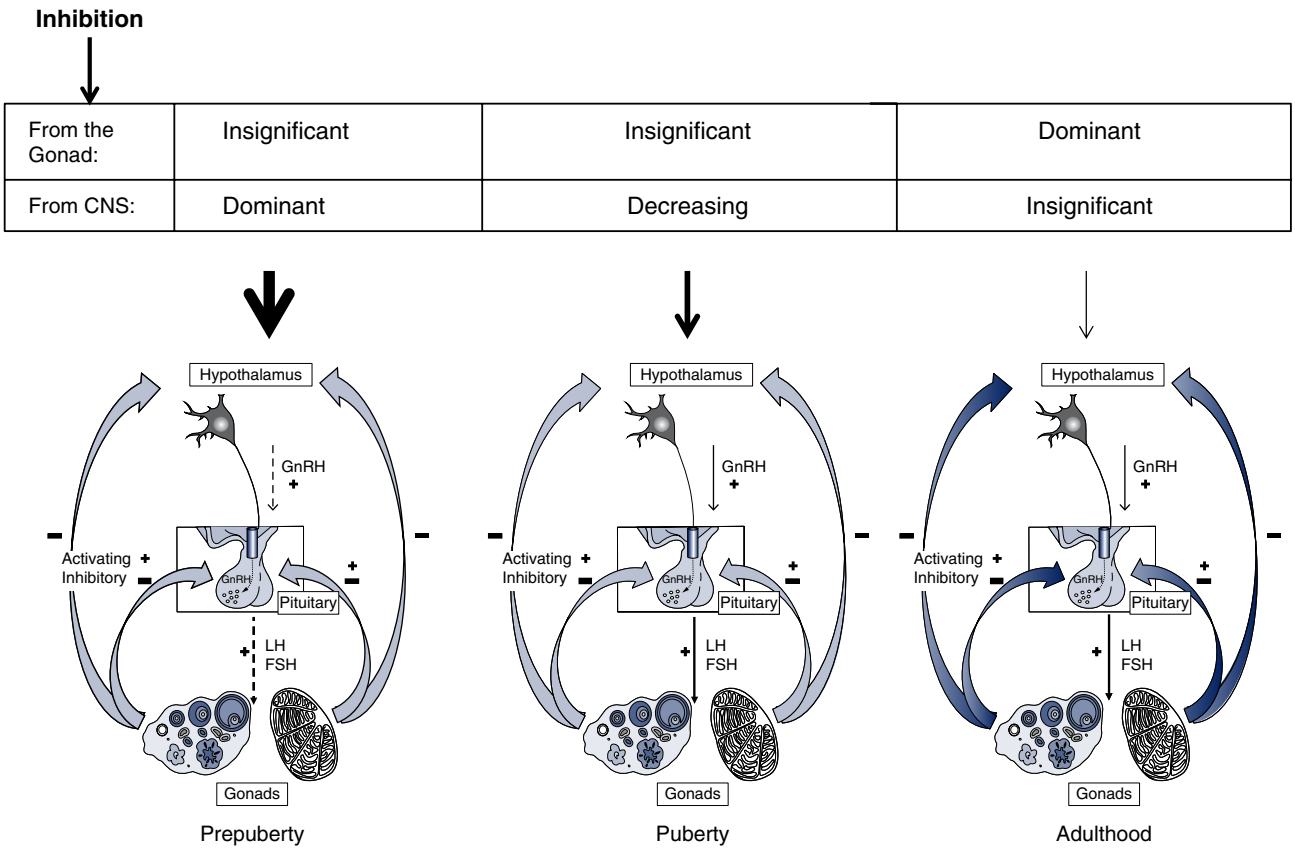

Figure 7.13 Inhibitory regulation of the hypothalamic–pituitary axis. This inhibitory regulation develops gradually after puberty onset, with reduction in the central suppressant drive from the CNS and increasing strength of feedback from the gonads.

puberty. The differences between diurnal and nocturnal concentrations of LH remain until the late stages of puberty but disappear by early adulthood. During this reactivation of the axis, there is a gradual development of a dynamic interplay between the central production of GnRH and gonadotropins and gonadal sex steroid production, with progressive maturation of negative and positive feedback loops. The central suppressant drive from the CNS gradually abates and intensifying positive feedback results from the increasing size of sex steroid production by the gonads. A gonadal contribution to the inhibition of the hypothalamic–pituitary system occurs later, becoming operative only at mid-puberty, and eventually becomes dominant over the central inhibitory feedback drive (Figure 7.13). Mean LH and FSH concentrations both increase through pubertal development, although LH concentration increases to a greater extent, probably due to differences in feedback mechanisms for the two hormones. The rises are due to both an increase in basal concentrations of LH and FSH and a greater number and amplitude of LH peaks.

In males during puberty, plasma testosterone concentrations increase dramatically. Table 7.3 summarizes the concentrations of testosterone at various developmental stages, with respective testicular sizes. The pubertal increase in testicular size results primarily from an increased number of proliferating and differentiating germ cells and, to a lesser extent, an increase in Sertoli cells. In early and mid-puberty, there is a pronounced diurnal rhythm with a morning peak in measureable testosterone but this is less pronounced in later puberty and declines gradually with age, probably due to decreased day–night ratios of gonadotropins.

The biological reactivation of the gonadotropic axis occurs earlier in girls than boys and the pattern of the reactivation of the gonadotropic axis is not identical in the two sexes. The secretion of testosterone increases shortly after the increase in the plasma concentration of LH and FSH. In girls, oestradiol increases together with increasing LH and FSH. A hormonal dialogue between gonads, hypothalamus and pituitary then contributes to the progressive activation of the gonadotropic axis until the end of puberty, which is marked by the occurrence of the LH ovulatory pulse in females. This sexual dimorphism may be related to the hormonal status. It could also be a feature of the sexual dimorphism of the brain.

Puberty is therefore the result of a neurodevelopmental process to attain homeostasis of the HPG axis.

Table 7.3 Pubertal stages (according to Tanner) with respective testis volumes and plasma testosterone concentrations.

Pubertal G-stage (Tanner)	Testicular volume (mL)	Plasma testosterone (ng/dL)	Plasma testosterone (nmol/L)
1	<4	<10	<0.3
2	4–8	12–69	0.4–2.4
3	8–10	60–275	2.1–9.5
4	10–20	142–515	4.9–17.9
5	20–25	319–775	11.1–26.9

Source: Values adapted from Knoor et al. [38].

Although somatic changes such as breast development or voice breaking are external indicators of sex steroid hormone levels, there is no currently clinically useful biomarker to date the neuroendocrine reactivation of the HPG axis.

Understanding the third activation of the gonadotropic axis at the end of the juvenile period remains incomplete but has advanced in some areas. GnRH neuronal activity is under the control of several neurotransmitters and neuropeptides, as described above, and the onset of puberty is triggered by a decline in these inhibitory signals and amplification of the excitatory inputs, leading to increased frequency and amplitude of GnRH pulses. However, the neuroendocrine mechanisms that control the first activation of the gonadotropic axis in the fetus, as well as the 'mini-puberty', remain unknown. Likewise, the exact mechanism of the juvenile break remains unknown.

Puberty is marked by the change of the balance of GABA–glutamate signalling in the brain. This is associated with a higher dendritic spine density and a simplification of the dendritic architecture of GnRH neurons. The timing of puberty is also correlated to an increase of the kisspeptin signalling in the hypothalamus that is due to an increase of kisspeptin synthesis as well as an increased responsiveness of GnRH neurons to kisspeptin stimulation. Although mainly described in mice, this paradigm is probably true in monkeys as well and relatively well conserved during evolution.

The mechanisms responsible for the increased biosynthesis of kisspeptins at the end of the juvenile period in the hypothalamus remain unknown. Data pointing to hypothalamic regulation via a hierarchical network of genes (Figure 7.11) have come mainly from a systems biology approach [39] and animal models [14] with little data from human subjects. Candidate transcriptional regulators identified by these approaches include *Oct-2*, *TTF-1* and *EAP1*. *Oct-2* is a transcriptional regulator of the POU domain family of homeobox-containing genes. *Oct-2* mRNA is upregulated in the hypothalamus in juvenile rodents; blockage of Oct-2 synthesis delays age at first ovulation and hypothalamic lesions that induce PP (e.g. hamartomas) activate Oct-2 expression. *TTF-1* (thyroid transcription factor-1) is another homeobox gene that enhances GnRH expression. *TTF-1* expression is increased in pubertal rhesus monkeys. *EAP1* mRNA levels also increase in the hypothalamus of primates and rodents during puberty, *EAP1* transactivates the *GnRH* promoter and EAP1 knockdown with siRNA caused DP and disrupted oestrous cyclicity in a rodent model.

Recent data have highlighted the importance of a genetic program that controls the expression of *Kiss1*. The intervention of the polycomb complex proteins EED and Cbx7 in the transcriptional repression of *Kiss1* has recently been revealed. The expression of these genes in the prepubertal period decreases with increasing methylation of their promoters. The binding of EED on the *Kiss1* promoter decreases at puberty. The inhibition of the repression of *Kiss1* is also correlated to a decrease in the expression of transcription factors with zinc finger motifs.

In addition, a microRNA switch was proposed to regulate the rise of *GnRH1* synthesis, which occurs during the juvenile period in GnRH neurons. The increase of kisspeptin expression in the hypothalamus therefore results from a complex network of transcription factors mainly acting as repressors of *Kiss1* and *GnRH1* transcription. The plasticity of GnRH neurons to kisspeptin stimulation is more obscure. The kisspeptin receptor is a G-protein-coupled receptor coupled to Gq/11 protein. The regulation of GPCR activity is multifactorial. GPCRs can undergo not only acute but also chronic desensitization through multiple intracellular signalling pathways and regulatory proteins. This regulation is supposed to be associated with the maturation status of GnRH neurons.

The concept that puberty results from the disappearance of gonadotropic axis repression is also supported by the description of loss-of-function mutations of *MKRN3* in familial CPP [40]. This gene encodes makorin ring finger protein 3, which not only can bind to RNA but could also be involved in ubiquitination. MKRN3 might

have an inhibitory effect on GnRH network since its expression in the ARC decreases in mice between birth and weaning. Where *MKRN3* might be placed in the hierarchical network of genes controlling kisspeptin has yet to be determined.

Puberty must thus be considered as the output of a neurodevelopmental programme that shares several features with the postnatal development of other neuronal functions. The specificity of this programme resides in its timing, which is determined by genetic factors dependent on the hormonal status and modulated by environmental factors. It is not surprising that an absence of puberty or incomplete pubertal development is not infrequent in humans.

Disturbances of puberty encompass an important group of pathologies within the field of pediatric endocrinology affecting over 4% of adolescents. In addition, abnormal timing of pubertal development is associated with adverse health and psychosocial outcomes. This not only has importance for the individual but also has a potential major impact on public health, especially in view of the secular trend towards an earlier age of puberty onset. Early puberty in particular is associated with adverse health outcomes, including breast and endometrial cancer, obesity, type 2 diabetes, cardiovascular disease, short stature and even increased mortality. Until recently it had not been clearly shown that late pubertal timing was also associated with adverse health outcomes but data from the UK Biobank study from both men and women has demonstrated that DP also has profound impacts on health in later life [41]. The considerable progress in understanding the mechanisms that control puberty over the last 10 years not only has been a success story of basic research in neuroendocrinology but also has been translated into clinical practice to allow a better understanding of the aetiopathogenic mechanisms of disordered pubertal development and, in some cases, to enable diagnostic genetic testing and counselling.

Precocious Puberty

Introduction

PP is defined as the onset of physical signs of puberty before the age of 8 years in girls and 9 in boys. Premature sexual development results from the action of sex steroids or compounds with sex steroid activity on target organs. PP leads to the progressive development of secondary sexual characteristics including breast development in girls and testicular enlargement in boys, together with the pubic hair, an acceleration of growth velocity and bone maturation resulting in premature fusion of the growth plates, potentially responsible for adult height deficit in these individuals.

The onset of puberty may be subject to the effects of environmental (secular trends, adoption, absence of the father and possible exposure to oestrogenic EDCs), nutritional (BMI) and constitutional (genetics, ethnicity) factors with implications for the definition of PP [2]. The consensus on the age limits for definition of PP has remained among European clinicians despite the observed trend towards a decreasing age of breast development in girls in the developed world. The female age cut-off is based on original data in white British girls; more recent data show that by this definition a significant minority of Afro-Caribbean girls in the USA will be classified as having PP. Premature development of pubic hair has also been shown in several studies to be more common in Afro-Caribbean children. Some centres have argued for a redefinition of the age cut-off for PP in girls to prevent over-investigation and treatment but concerns remain that cases due to pathological causes of CPP in 7–8-year-old girls will be missed.

Premature sexual maturation is a frequent cause for referral. Clinical evaluation is generally sufficient to reassure the patients and their families but premature sexual maturation may reveal severe conditions and thorough evaluation is therefore required to identify its cause and potential for progression so that appropriate treatment can be proposed.

The phenotype of PP is polymorphic. In addition to progressive CPP, with a progressive deterioration of adult height prognosis in the absence of treatment, there are very slowly progressive forms that do not modify predicted final height. It is not always straightforward to recognize these different clinical forms at the initial evaluation. Nevertheless, their correct identification is important because the indicated treatments differ. The heterogeneity of PP in terms of its clinical presentation and definition can be explained by the gradual nature of the transition to puberty.

As discussed above, the pulsatile secretion of LH begins before the onset of clinical signs of puberty and an increase in the amplitude of the LH peaks is the key biological sign of pubertal maturation of the HPG axis. GnRH stimulation tests indirectly reflect pulsatile endogenous GnRH secretion, as this secretion determines the response to exogenous GnRH. The available data indicate that there is no clear boundary between prepubertal and pubertal status accounting for the frequency of 'marginal' forms of PP.

Aetiologies of Premature Sexual Development

Central Precocious Puberty

PP is categorized into central (gonadotropin-dependent) causes involving activation of hypothalamic GnRH pulsatility or peripheral (gonadotropin-independent)

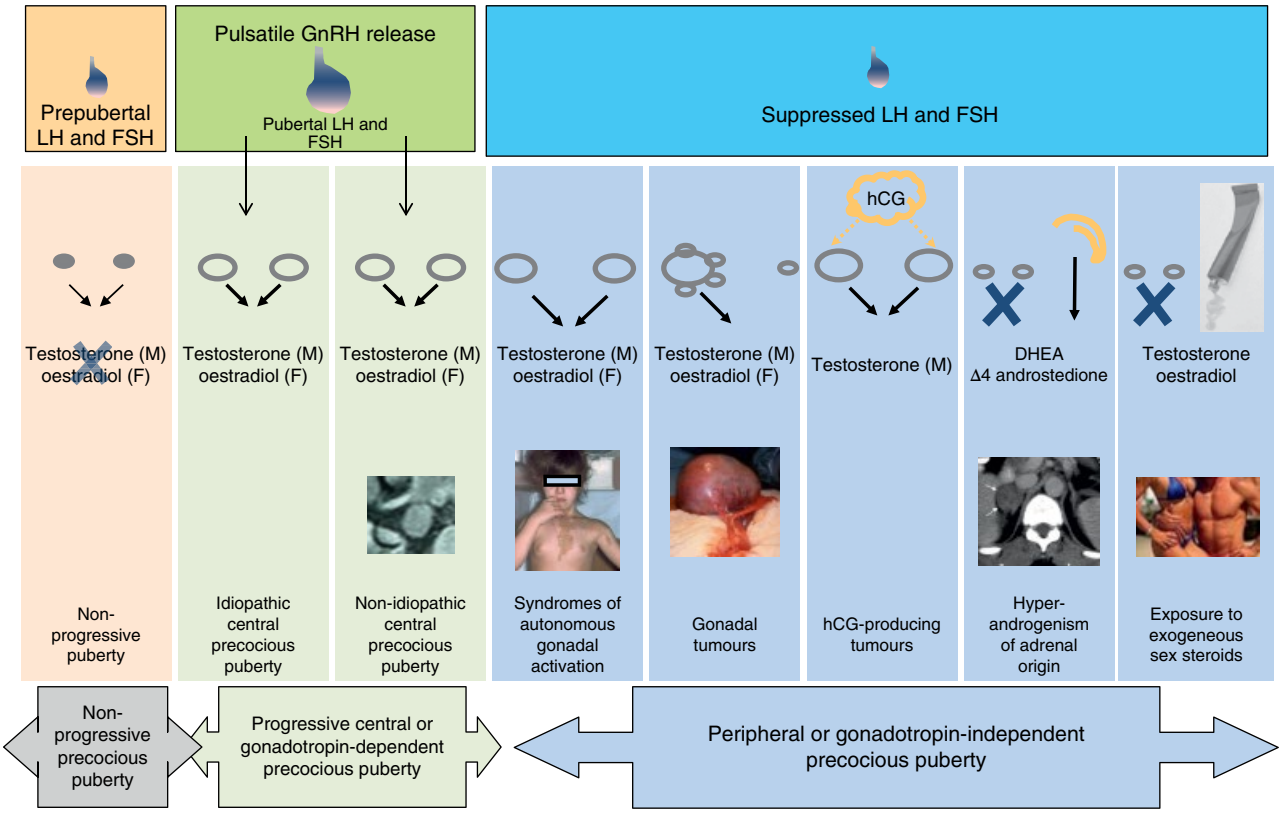

Figure 7.14 Aetiologies of precocious puberty. (*See insert for colour representation of the figure.*)

comprising a group of conditions that result from autonomous activation of gonadal hormone production (Figure 7.14). Diagnostic work-up must differentiate CPP from peripheral PP. CPP results from premature reactivation of the HPG axis and pulsatile GnRH secretion with a hormonal pattern similar to that of normal puberty. Puberty progression in CPP is symmetrical and the endocrine and physical events occur in the same pattern and at the same pace as normally timed puberty (i.e. consonant), while in peripheral PP there may be asynchronous development of Tanner stages. In most girls, there is no CNS defect identified and CPP is defined as idiopathic. Precocious onset of puberty is ~5 times more common in girls than boys. The prevalence of CPP in girls was found to be 0.2% but only 0.05% in boys over a 9-year period in one European series [42].

The underlying reasons for this gender difference are not clear but an underlying abnormality is found far more commonly in boys, suggesting that many cases of female PP may represent the end of the normal spectrum without underlying pathology. Additionally, it appears that the female HPG axis may be more sensitive to environmental factors such as increased fat mass than the male. This is seen also in populations with functional hypogonadism due to weight loss or excessive exercise, where more women than men are affected. A small trend towards earlier testicular development in boys with increased BMI has also been found and, possibly related to this, an increase in boys with idiopathic PP without an organic cause has been observed recently. Despite this, it is important to be mindful of excluding an underlying pathological cause in both genders. The earlier the age of presentation, the more likely it is to identify an organic cause (Table 7.4) [2].

CPP may be due to tumours including astrocytomas, gliomas and germ cell tumours or other hypothalamic lesions including hamartomas or congenital abnormalities including arachnoid cysts, which may be identified on MRI. Hypothalamic hamartomas may be associated with a typical pattern of epilepsy (gelastic seizures). All conditions that give rise to damage to the hypothalamic–pituitary axis can represent a risk factor for CPP, including radiotherapy to the hypothalamus or pituitary for pediatric cancers, hydrocephalus, birth asphyxia or neurodegenerative disease. CPP and early puberty are common in otherwise healthy girls adopted from developing countries and may be more common after psychological distress including sexual abuse. A number of dysmorphic syndromes are associated with CPP, for example, Kabuki syndrome.

Table 7.4 Clinical characteristics of the various forms of central precocious puberty.

Cause	Symptoms and signs	Evaluation
Due to a CNS lesion		
Hypothalamic hamartoma	May be associated with gelastic (laughing seizures), focal or tonic–clonic seizures	MRI: mass in the floor of the third ventricle isointense to normal tissue without contrast enhancement
Other hypothalamic tumours: • Glioma involving the hypothalamus and/or the optic chiasm • Astrocytoma • Ependymoma • Pinealoma • Germ cell tumours	May include headache, visual changes, cognitive changes, symptoms/signs of anterior or posterior pituitary deficiency (e.g. decreased growth velocity, polyuria/polydipsia), fatigue, visual field defects If CNS tumour (glioma) associated with neurofibromatosis, may have other features of neurofibromatosis (cutaneous neurofibromas, café au lait spots, Lisch nodules, etc.)	MRI: contrast-enhanced mass that may involve the optic pathways (chiasm, nerve, tract) or the hypothalamus (astrocytoma, glioma) or that may involve the hypothalamus and pituitary stalk (germ cell tumour), may have evidence of intracranial hypertension May have signs of anterior or posterior pituitary deficiency (e.g. hypernatraemia) If germ cell tumour: ßhCG detectable in blood or CSF
Cerebral malformations involving the hypothalamus: • Suprasellar arachnoid cyst • Hydrocephalus • Septo optic dysplasia • Myelomeningocele • Ectopic neurohypophysis	May have neurodevelopmental deficits, macrocrania, visual impairment, nystagmus, obesity, polyuria/polydipsia, decreased growth velocity	May have signs of anterior or posterior pituitary deficiency (e.g. hypernatraemia) or hyperprolactinaemia
Acquired injury: • Cranial irradiation • Head trauma • Infections • Perinatal insults	Relevant history Symptoms and signs of anterior or posterior pituitary deficiency may be present	MRI may reveal condition-specific sequelae or may be normal
Idiopathic: no CNS lesion	≈ 92% of girls and ≈ 50% of boys History of familial precocious puberty or adoption may be present	No hypothalamic abnormality on the head MRI. The anterior pituitary may be enlarged MKRN3 gene evaluation if paternally transmitted
Secondary to early exposure to sex steroids		
After cure of any cause of gonadotropin-independent precocious puberty	Relevant history	

Like self-limited delayed puberty (DP), CPP often has a strong familial basis. Segregation analysis from one study suggested an autosomal dominant inheritance pattern with incomplete sex-dependent penetrance [43]. This study demonstrated a significantly earlier age of maternal menarche and a more advanced pubertal staging at presentation in the familial cohort as compared with sporadic CPP but few genes have been identified as causal in CPP. A heterozygous activating mutation in the *KISS1R* gene has been identified in one Brazilian girl with CPP. Rare variants in the *KISS1* gene have been found in a small number of children with CPP but mutations in these genes have not been found in other cohorts with familial CPP.

Recent studies have implicated the inactivation of makorin ring finger 3 (*MKRN3*) genes in 'idiopathic' CPP [40]. *MKRN3* is an imprinted gene located on the long arm of chromosome 15, which is involved in ubiquitination and cell signalling. *MKRN3* has been identified as an important gatekeeper in pubertal onset with a potentially inhibitory effect on GnRH secretion. *MKRN3* gene defects have been identified as a cause of paternally transmitted familial CPP but such defects do not underlie maternally transmitted CPP. Unlike *KISS1* and *KISS1R*, mutations in this gene in familial CPP appear to be relatively common in both genders, being identified in 33–46% of probands as compared to 3–6% of patients with sporadic CPP. This has not been verified in all populations. Mutations in *MKRN3* appear to be associated with an earlier age of puberty onset in girls as compared with boys, in keeping with a female HPG axis being more 'sensitive' to disruption by genetic or environmental factors.

The median age of CPP at 6 years in girls with *MKRN3* mutations was not as extreme as with, for example, the *KISS1* mutations that have been identified. This has led to the suggestion that the action of *MKRN3* may not be required to suppress the activation of the HPG axis in early childhood but instead is important for regulation of pubertal timing in the later prepubertal period. Abreu et al. demonstrated a prominent reduction in *MRKN3* mRNA expression in the ARC of mice immediately before puberty. Together this adds weight to the hypothesis that *MRKN3* may be part of the mechanism to release the inhibitory 'brake' at the onset of puberty. More recently, a paternally inherited *DLK1* deletion has been reported in one family with isolated CPP [44].

Peripheral Precocious Puberty

Peripheral or gonadotropin-independent PP is due to the production of sex steroids by gonadal or adrenal tissue independently of gonadotropins (which are generally supressed). Peripheral PP can result from gonadal, adrenal or hCG-producing tumours (in boys) and exposure to exogenous sex steroids or compounds with steroidal activity. Male-limited PP or familial male PP is due to an autosomal dominant inherited activating mutation in the LH receptor, which presents in males at a young age with penile and testicular growth, advanced bone age and sexualized behaviour (Table 7.5). Peripheral PP can rarely lead to activation of pulsatile GnRH secretion and to CPP due to prolonged priming of the HPG axis (Table 7.4).

In addition to these causes, peripheral PP may also be seen in McCune–Albright syndrome (MAS), aromatase inhibitor excess and congenital adrenal hyperplasia (CAH). While classical CAH more commonly presents in the newborn period, non-classical CAH can present as precocious pubarche (development of pubic hair) with accelerated bone growth [45]. MAS is a predominant cause for peripheral PP in females. This condition, involving genetic mosaicism, is caused by somatic cell (post-zygotic) mutations in the Gsα subunit (*GNAS1* gene) of the Gs protein that activates adenylate cyclase. This activating mutation can result in multiple endocrinopathies, with PP occurring due to effects on LH receptor function with constitutive activation and sex steroid production in the absence of LH binding. Early pubertal development in MAS is common, often at 2–3 years of age. The condition also includes polyostotic fibrous dysplasia of the bone and patches of skin pigmentation with a characteristic 'coast of Maine' border.

The pattern of pubertal development in peripheral PP may be asynchronous, unlike in normal puberty or CPP, for example, with menarche occurring at an early stage of breast development. Autonomous ovarian cysts may present with vaginal bleeding and breast development. Hypothyroidism associated with ovarian cysts can present in a similar way.

Consequences of Precocious Puberty

Progressive premature sexual maturation can have consequences on growth and psychosocial development. Growth velocity is accelerated as compared to normal values for age and bone age is advanced in most cases. The acceleration of bone maturation can lead to premature fusion of the growth plate and short stature. Several studies have assessed adult height in individuals with a history of PP. In older published series of untreated patients, mean heights ranged from 151 to 156 cm in boys and 150 to 154 cm in girls, corresponding to a loss of about 20 cm in boys and 12 cm in girls relative to normal adult height [46]. These numbers correspond to historical series of patients with severe early onset PP, which are not representative of the majority of patients seen in the clinic today. Height loss due to PP is inversely correlated with the age at pubertal onset and currently treated patients tend to have a later onset of puberty than those in historical series.

Parents often seek treatment in girls because they fear early menarche, but there are few data to predict the age of menarche following early onset of puberty. In the general population, the time from breast development to menarche is longer for children with an earlier onset of puberty, ranging from a mean of 2.8 years when breast development begins at age 9 to 1.4 years when breast development begins at age 12.

In the general population, early pubertal timing has been shown to be associated with several adverse health outcomes in adult life with higher risks for cardiovascular disease and type 2 diabetes in both women and men [41] (see 'The hypothalamic–pituitary–gonadal axis' section). There are no long-term data on these aspects in the case of PP.

Adverse psychosocial outcomes are also a concern but the available data specific to patients with PP have serious limitations. In the general population, a higher proportion of early-maturing adolescents engage in exploratory behaviours (sexual intercourse, legal and illegal substance use) and at an earlier age than adolescents maturing within the normal age range or later. In addition, the risk for sexual abuse seems to be higher in girls or women with early sexual maturation but the relevance of these findings to PP is unclear and they should not be used to justify intervention.

Evaluation of the Child with Premature Sexual Development

The evaluation of patients with premature sexual development should address several questions: (1) Is sexual

Table 7.5 Clinical characteristics of the various forms of peripheral precocious puberty.

Disorder	Characteristic symptoms and signs	Test results
Autonomous gonadal activation		
McCune–Albright syndrome and recurrent autonomous ovarian cysts due to somatic activating mutation of the *GNAS* gene resulting in increased signal transduction in the Gs pathway	Mostly in girls. Typically rapid progression of breast development and early occurrence of vaginal bleeding (before or within a few months of breast development) Precocious puberty may be isolated or associated with café au lait pigmented skin lesions or bone pain due to polyostotic fibrous dysplasia. More rarely other signs of endocrine hyperfunction (e.g. hypercortisolism, hyperthyroidism), liver cholestasis or cardiac rhythm abnormalities	Typically large ovarian cyst or cysts on pelvic ultrasound examination Bone lesions of fibrous dysplasia May have laboratory evidence of hypercortisolism, hyperthyroidism, increased GH secretion, hypophosphataemia, liver cholestasis
Familial male-limited precocious puberty due to germline-activating mutations of the LH receptor gene	A familial history of dominant precocious puberty limited to boys (but transmitted by mothers) may be present but some cases are sporadic	Activating mutation of the LH receptor gene
Germline mutations of *GNAS* gene, resulting in dual loss and gain of function (rare)	Single case report of a boy with concomitant pseudohypoparathyroidism and gonadotropin-independent precocious puberty	
Tumours		
Granulosa cell tumours of the ovary	Rapid progression of breast development; abdominal pain may occur. The tumour may be palpable on abdominal examination	Tumour detection on ultrasound or CT scan
Androgen-producing ovarian tumours	Progressive virilization	Tumour detection on ultrasound or CT scan
Testicular Leydig cell tumours	Progressive virilization; testicular asymmetry (the tumour itself is rarely palpable)	Tumour detection on testicular ultrasound
hCG-producing tumours	Tumours can originate in the liver or mediastinum. Pubertal symptoms in boys only. May be associated with Klinefelter syndrome	Elevated serum hCG
Adrenal disorders	Manifest with signs of androgen exposure	
Congenital adrenal hyperplasia	Increased androgen production, leading to virilization in boys and girls	Increased adrenal steroid precursors in serum, mainly 17OH-progesterone (basal or after an ACTH stimulation test)
Adrenal tumour	Increased androgen production, leading to virilization in boys and girls. Very rarely, oestrogen-producing adrenal tumour	Tumour on abdominal ultrasound or CT scan Elevated DHEAS or adrenal steroid precursors
Generalized glucocorticoid resistance	Symptoms and signs of mineralocorticoid excess, such as hypertension and hypokalaemic alkalosis	Elevated urinary free cortisol and plasma cortisol
Environmental agents		
Exogenous sex steroids	Manifestations vary with the type of preparation (androgenic or oestrogenic); most commonly described after topical exposure to androgens; tracing the source of exposure may be difficult	Endocrine evaluation can be misleading due to widely variable serum levels of sex steroids with time
Exposure to oestrogenic endocrine-disrupting chemicals	May play a role in precocious puberty in adopted children (by modulating the timing of pubertal gonadotrophic axis activation) although this remains unproven	No validated biochemical test
Severe untreated primary hypothyroidism	Signs of hypothyroidism. No increase of growth velocity Manifest mostly with increased testicular volume in the absence of virilization. Due to a cross-reactivity of elevated TSH to the FSH receptor	Elevated serum TSH concentration, low free T4 concentration. No bone age advancement

development really occurring outside the normal temporal range? (2) What is the underlying mechanism and is it associated with a risk of a serious condition, such as an intracranial lesion? (3) Is pubertal development likely to progress? (4) Would this impair the child's normal physical and psychosocial development?

Clinical Diagnosis

PP manifests as the progressive appearance of secondary sexual characteristics – breast development, pubic hair and menarche in girls and enlargement of testicular volume (testicular volume >3 mL) and penile and pubic hair development in boys (Figure 7.2) – together with an acceleration of height velocity and bone maturation, which is frequently very advanced (by more than two years relative to chronological age). A single sign may remain the only sign for long periods, making diagnosis difficult, particularly in girls, in whom isolated breast development may precede the appearance of pubic hair or the increase in growth velocity and bone maturation by several months. In some children, the increase in height velocity precedes the appearance of secondary sexual characteristics. Clinical evaluation should guide diagnosis and discussions about appropriate management (Table 7.6).

Table 7.6 When should suspected precocious puberty be explored?

Girls	Boys
Breast development before the age of 8 years	Increase in testicular volume before the age of 9 years
Pubic hair before the age of 8 years	Pubic hair before the age of 9 years
Breast development between the ages of 8 and 9 years. Exploration only if: • Onset of pubertal development before 8 years • Growth velocity > 6 cm/year, adult height prognosis below target height • Rapid progression of pubertal development (transition from one stage to another in <6 months) • Clinical evidence for a neurogenic aetiology • Clinical evidence of peripheral precocious puberty	Pubertal development seen around 10 years. Exploration only if: • Onset of pubertal development before 9 years • Growth velocity > 6 cm/year, adult height prognosis below target height • Rapid progression of pubertal development (transition from one stage to another in <6 months) • Clinical evidence of a neurogenic aetiology • Clinical evidence of peripheral precocious puberty
Menarche before the age of 10 years	

History taking is used to specify the age at onset and rate of progression of pubertal signs to investigate neonatal parameters (gestational age, birth measurements) and whether the child was adopted, together with any evidence suggesting a possible CNS disorder, such as headache, visual disturbances or neurological signs (gelastic seizures); pituitary hormone deficiency, such as lethargy, polyuria/polydipsia and the existence of a known chronic disease; or history of cerebral radiotherapy. Evaluation also includes heights and pubertal ages of parents and siblings and family history of early or advanced puberty.

Physical examination should assess height, height velocity, weight and BMI, pubertal stage and, in girls, the oestrogenization of the vulva, skin lesions suggestive of neurofibromatosis or MAS, neurological signs (large head circumference with macrocephaly, nystagmus, visual change or visual field defects, neurodevelopmental deficit) and symptoms or signs of anterior or posterior pituitary deficiency (low growth velocity, polyuria/polydipsia, fatigue). The neuropsychological status of the child should also be assessed because it may be the major concern of the child and parents seeking help for early puberty. It is important to recognize clinically the benign variants of precocious pubertal development usually involving the isolated and non-progressive development of secondary sexual characteristics (breasts or pubic hair), normal growth velocity or slight increase in growth velocity with little or no bone age advance.

Following this assessment, watchful waiting or complementary investigations may be chosen as the most appropriate course of action. The criteria used to guide explorations are presented in Table 7.6. If watchful waiting is chosen, re-evaluation of progression is required three to six months later to assess the rate of progression of puberty and changes in growth.

Additional testing is generally recommended in all boys with precocious pubertal development, in girls with precocious Tanner 3 breast stage or higher and in girls with precocious B2 stage and additional criteria, such as increased growth velocity or symptoms or signs suggestive of CNS dysfunction or of peripheral PP. These tests include assessment of bone age (which is usually advanced in patients with progressive PP), biochemical investigations including sex steroids and gonadotropins, pelvic or testicular (if peripheral PP is suspected) ultrasound scans and brain MRI.

Peripheral PP is independent of the hypothalamic–pituitary axis, with high serum oestradiol concentrations in girls and high serum testosterone concentrations in boys, low basal and peak serum LH concentrations after GnRH stimulation, advanced bone age and an oestrogenized uterus on ultrasound scan for girls. The gonads or adrenal glands are responsible for excess steroid

production but they may also promote the activation of pubertal maturation of the gonadal axis, resulting in CPP. Measurement of hCG, alpha fetoprotein and other tumour markers is warranted if peripheral PP is suspected.

Biological Diagnosis

The biological diagnosis of PP is based on the evaluation of sex steroid secretion and its underlying mechanism. The diagnosis of CPP is based on pubertal serum gonadotropin concentrations with the demonstration of activation of gonadotropin secretion.

In boys, testosterone is a good marker of testicular maturation, provided it is assessed with a sensitive method. Radioimmunoassay (RIA) is generally used in practice. In girls, oestradiol determination can be uninformative because half the girls with CPP have oestradiol concentrations within the normal range of values for prepubescent girls but markedly raised oestradiol concentrations may be seen in MAS or ovarian disease due to cysts or tumours. Very sensitive methods are required and only RIA meets this requirement. The increase in oestradiol concentration is also highly variable due to the fluctuation and sometimes intermittent secretion of this hormone. Oestrogenic exposure is best assessed by pelvic ultrasound scans when oestrogenization of the uterus and ovaries may be visible.

Basal gonadotropin concentrations are informative and are generally significantly higher in children with PP than in prepubertal children but basal serum LH concentration is much more sensitive than basal FSH concentration and is the key to diagnosis. Ultrasensitive assays such as a chemiluminescent immunometric assay should be used to determine serum LH concentration. Prepubertal LH concentrations are <0.1 IU/L, so LH assays should have a detection limit close to 0.1 IU/L. The response to GnRH stimulation is considered the gold standard for the diagnosis of CPP. Stimulation tests involving a single injection of LHRH analogues can also be used.

The major problem is defining the decision threshold. In both sexes, a central cause of PP is demonstrated by an increase in pituitary gonadotropin concentrations. Indeed, the underlying mechanism of early central puberty is linked to premature activation of the HPG axis, with the onset of pulsatile LH secretion and an increase in the secretion of pituitary gonadotropins both in the basal state and after stimulation with LHRH. Before the onset of puberty, the FSH peak is greater than the LH surge. During and after puberty, the LH surge predominates. In cases of CPP, basal serum LH concentration usually is ≥ 0.3 IU/L and serum LH concentration after stimulation is ≥5 IU/L [47]. FSH is less informative than LH, because FSH concentrations vary little during pubertal development but the stimulated LH/FSH ratio may make it easier to distinguish progressive PP (with an LH/FSH ratio of >0.66) from non-progressive variants not requiring GnRHa therapy.

Place of Imaging in the Evaluation of Precocious Puberty

Pelvic ultrasound scans can be used to assess the degree of oestrogenic exposure of the internal genitalia in girls through measurements of size and morphological criteria. A uterine length of ≥35 mm is the first sign of oestrogen exposure. Morphological features are also important, as the prepubertal state is marked by a tubular uterus, which becomes more pear-like in shape during the course of puberty with a bulging fundus. Measurements of uterine volume increase the reliability of the examination (prepubertal ≤ 2 mm). Endometrial thickening on an endometrial ultrasound scan provides a second line of evidence. Menarche usually requires an endometrium of about 8 mm.

Ovary size and the number of follicles are not criteria for the assessment of pubertal development but ovarian volume and number of follicles will increase over the course of pubertal maturation. Testicular ultrasound should be performed if the testes differ in volume or if peripheral PP is suspected to detect Leydig cell tumours, which are generally not palpable but can be suspected based on testicular calcification or discrepancy in testicular volumes.

Neuroimaging is essential in the aetiological evaluation of progressive CPP. MRI of the brain is the examination of choice for detection of CNS tumours and lesions in the hypothalamic–pituitary region. The prevalence of such lesions is higher in boys (30–80% of cases) than in girls (8–33%) and is much lower when puberty starts after the age of 6 years in girls, which accounts for the majority of cases. It has been suggested that an algorithm based on age and oestradiol concentrations could replace MRI but such an approach has not been validated.

The Normal Variants of Early Pubertal Signs

The distinction between early and normal puberty is not clear-cut. There are several variants of normal puberty that may pose problems for differential diagnosis, particularly as they have a high prevalence. Cases of premature sexual maturation that correspond to variants of normal development can mimic PP but do not lead to long-term consequences and are usually benign. This is particularly true of girls below the age of 2–3 years where the condition is known as premature thelarche. Similarly, in older girls, at least 50% of cases of premature sexual maturation will regress or stop progressing and no treatment is necessary [48]. Therapeutic abstention is the most appropriate approach in most of these cases

Table 7.7 Differentiation between true precocious puberty and slowly progressive forms.

		Progressive precocious puberty	Slowly progressive precocious puberty
Clinical signs	Pubertal stage	Passage from one stage to another in 3–6 months	Spontaneous regression or stabilization of pubertal signs
	Growth velocity	Accelerated: >6 cm/year	Normal for age
	Bone age	Typically advanced, variable, at least 2 years	Variable, but usually within 1 year of chronological age
	Predicted adult height	Below target height or decreasing on serial determinations	Within target height range
Pelvic ultrasound scan	Uterus	Length > 34 mm or volume > 2 mL	Length ≤ 34 mm or volume ≤ 2 mL
		Pearl-shaped uterus	Prepubertal, tubular uterus
		Endometrial thickening (endometrial ultrasound scan)	
	Ovaries	Not very informative	Not very informative
Hormonal evaluation	Oestradiol	Not very informative, usually measurable	Not detectable or close to the detection limit
	LH peak after stimulation with GnRH	In the pubertal range of ≥5 IU/L	In the prepubertal range
	Basal LH determination	Useful if value is high (≥3 IU/L) and frankly in the pubertal range	No definitive value

because puberty progresses slowly, with menarche occurring on average of 5.5 years after the onset of clinical signs of puberty and patients reaching a normal final height relative to parental target height.

The diagnostic approach outlined above should help determine the progressive or non-progressive nature of pubertal precocity (Table 7.7) and differentiate between the aetiologies of central or peripheral PP but, in about one-third of subjects, predicted adult stature may decrease during the progression of puberty in parallel with the emergence of evident biological signs of oestrogenization and a progressive form of CPP. Thus children for whom no treatment is justified at the initial assessment should follow up with serial clinical assessments at least until the age of 9 years to facilitate the identification of girls subsequently requiring medical treatment to block CPP.

Isolated Premature Breast Development or Premature Thelarche

Premature thelarche is isolated breast development before the age of 8 years. There are two peaks in the frequency of premature thelarche, the neonatal period, which is marked by gonadotropin activation potentially lasting for 2 or 3 years, and the prepubertal period. Premature thelarche differs from early puberty in the absence of any other aspect of sexual development, usually with only early Tanner stage breast development and no acceleration of height velocity or significant advance in bone maturation (≥2 years) [49]. Premature thelarche probably represents an exaggerated form of the physiological early gonadotropin surge that is delayed in girls relative to boys.

The principal endocrine feature is a dominance of pulsatile FSH secretion, which stimulates low levels of oestrogen secretion. Uterine ultrasound scans provide a simple means of checking that there is no change in the uterus. No further investigation or treatment is required and the natural history of this variant is the persistence of moderate breast development in two-thirds of cases or regression in one-third of cases. Isolated premature breast development may precede the onset of CPP, which should not be ignored if patients develop other pubertal signs and/or an acceleration of height velocity.

Premature Development of Pubic Hair or Premature Pubarche

Premature pubarche (or adrenarche) is the appearance of pubic hair (and axillary hair) before the age of 8 years in girls and nine years in boys. It corresponds to adrenal maturation (adrenarche) and is not a differential diagnosis for CPP. While adrenarche is normally temporally coordinated with pubertal development, it is not required for it nor dependent on it. Indeed, production of adrenal androgens including dehydroepiandrosterone (DHEAS) and androstenedione from the zona reticularis increases two years or more before gonadotropins and sex steroids increase. ACTH appears to be necessary for adrenarche but is not the sole stimulus.

Premature adrenal androgen production can stimulate apocrine sweat glands and the development of body odour and can be associated with a slightly accelerated

growth rate or advance in bone age with modest signs of hyperandrogenism. Endogenous puberty occurs at a normal time and adult height is within the expected range for parents. It is seen more commonly in children born small for gestational age. Possible differential diagnoses to be excluded in a child with precocious development of pubic hair associated with accelerated growth, advanced bone age or acne, especially if other signs including clitoromegaly, voice deepening or extreme hirsutism are present, include adrenal tumours and CAH.

Slowly Progressive Forms of Precocious Puberty

These forms present clinically in girls as early puberty with the development of secondary sexual characteristics and a moderate advance in bone age, and the girls are on a clinical spectrum between true CPP and premature thelarche. On ultrasound scan, the uterus may show early oestrogen impregnation but the response to GnRH is prepubertal. The mechanism underlying these cases of non-progressive PP is unknown but the gonadotropic axis is not activated. Studies monitoring the benign variants of PP have shown that treatment with GnRH agonists is not appropriate because there tends to be either a total regression of pubertal signs or only a slow progression towards puberty. In keeping with this, a normal adult height close to parental target height is achieved. Table 7.7 provides guidance on the differentiation between slowly progressive and progressive forms of CPP. There is no male equivalent of premature thelarche or slowly progressive variations of PP.

Psychosocial Aspects

Psychosocial aspects of early puberty are a major concern of patients and families seeking help for early puberty, whereas doctors generally focus on aetiological aspects and height prognosis. Psychological assessment usually reveals a normal IQ. Patients tend to be rather solitary, with high scores for isolation and a tendency to become depressed. They are mostly concerned about their appearance, whereas parents are generally worried about the onset of periods. Although early menarche is associated with earlier advanced sexual experiences, long-term psychosocial outcomes of early normal puberty appear to be similar to women with average or late timing of puberty, although data on those individuals with very early PP and those treated for PP are limited [50].

Management

Central Precocious Puberty

GnRH Agonists

GnRH agonists are generally indicated in progressive CPP with the aim of restoring genetic growth potential and arresting or regressing pubertal symptoms. GnRH agonists continuously stimulate the pituitary gonadotrophs, leading to desensitization and decreases in LH release and, to a lesser extent, FSH release. Several GnRH agonists are available in various depot forms and the approval for use of the various formulations varies with countries. Despite nearly 30 years of use of GnRH agonists in PP, there are still ongoing questions on their optimal use and an international consensus statement has summarized the available information and the areas of uncertainty as of 2007 [51].

GnRH agonist regimens should be managed by experienced clinicians and treatment should result in the regression or arrest of pubertal symptoms, with a decrease in growth velocity and bone age advance. GnRHa injection dates should be recorded and adherence with the dosing interval monitored. A suppressed LH response to stimulation by GnRH, GnRH agonist or after an injection of the depot preparation (which contains a fraction of free GnRH agonist) is indicative of biochemical efficacy of the treatment but is not recommended routinely. Progression of breast or testicular development usually indicates poor compliance, treatment failure or incorrect diagnosis and requires further evaluation.

There are no randomized controlled trials assessing long-term outcomes of the treatment of CPP with GnRH agonists, and height as an outcome has been mostly assessed from observational data [52]. Among ~400 girls treated until a mean age of 11 years, the mean adult height was ~160 cm and mean gains over predicted height varied from 3 to 10 cm. Individual height gains were very variable but were calculated using predicted height, which is itself of limited accuracy. Factors affecting height outcome include initial patient characteristics (lower height if bone age is markedly advanced with shorter predicted height at initiation of treatment) and, in some series, duration of treatment (higher height gains in patients starting treatment at a younger age and with longer duration of treatment). No height gain benefit has been shown in girls treated after the age of 9 years.

Other outcomes to consider include bone mineral density, risk of obesity and metabolic disorders and psychosocial outcomes. Bone mineral density may decrease during GnRH agonist therapy but subsequent bone mass accrual is preserved and peak bone mass is not negatively affected by treatment. There has been concern that GnRH agonists use may affect BMI. Childhood obesity is associated with earlier pubertal development in girls and early sexual maturation is associated with increased prevalence of overweight and obesity. Altogether, the available data indicate that long-term GnRH agonist treatment does not seem to cause or aggravate obesity or have repercussions for body composition, bone mineral density, fertility and metabolic or cancer comorbidities. General health status is not different as compared with

women with normal puberty. The development of polycystic ovarian syndrome remains controversial and further studies are required to assess the potential risk of premature ovarian dysfunction. Data concerning psychosocial outcomes are scarce and there is little evidence to show whether treatment with GnRH agonists is associated with improved psychological outcome. Studies of this aspect are required.

Although tolerance to GnRH agonist treatment is generally considered good, it may be associated with headaches and menopausal symptoms such as hot flushes. Local complications such as sterile abscesses seen in 3–13% may result in a loss of efficacy, and anaphylaxis has been described.

The best time to stop treatment has not been established and factors that could influence the decision to stop GnRH agonists include aims of maximizing height, synchronizing puberty with peers, ameliorating psychological distress or facilitating care of the developmentally delayed child. Data only permit analysis of factors that affect adult height. Several variables can be used to decide on when to stop treatment including chronological age, duration of therapy, bone age, height, target height and growth velocity but these variables are closely interrelated and cannot be considered independently. Retrospective analyses suggest that continuing treatment beyond the age of 11 years is associated with no further gains. It is reasonable to consider these parameters and informed parent and patient preferences with the goal of menarche occurring near the population norms [51]. Pubertal manifestations generally reappear within months of GnRH agonist treatment being stopped, with a mean time to menarche of 16 months. Long-term fertility has not been fully evaluated, but preliminary observations are reassuring.

The addition of GH or oxandrolone when growth velocity decreases or if height prognosis appears to be unsatisfactory has been proposed but data are limited on the efficacy and safety of these drugs in children with PP.

Management of Causal Lesions

When PP is caused by a hypothalamic lesion (e.g. mass or malformation), management of the causal lesion generally has no effect on the course of pubertal development. Hypothalamic hamartomas should not be treated by surgery for the management of CPP. CPP associated with the presence of a hypothalamic lesion may progress to gonadotropin deficiency.

Peripheral Precocious Puberty

Management of Causal Lesions

Surgery is indicated for gonadal tumours and post-operative chemo- or radiotherapy should be discussed as part of a multidisciplinary team including surgeons and oncologists.

Large ovarian cysts (>20 mL or 3.4 cm in diameter and typically more than 75 mL or 5.2 cm) should be managed carefully given the risk of adnexal torsion. In such cases, ultrasound-guided biopsy should be considered, which allows molecular analysis of the cystic fluid for an activating GNAS mutation underlying MAS.

Removal of exogenous exposure to sex steroids is obviously required, but the search for occupational exposure is often very difficult and requires careful investigation.

Medications

There is no aetiological treatment for peripheral causes of PP and the rarity of the diseases renders evaluation of therapeutic strategies very difficult. In MAS and recurrent ovarian cysts, AIs and selective oestrogen receptor modulators (SERMs) (e.g. tamoxifen) have been used to inhibit the production or action of oestrogens [53]. These approaches are partially effective but no definitive strategy has emerged. In familial male PP due to LH receptor activating mutations, ketoconazole, an inhibitor of androgen biosynthesis, has been shown to be effective in the long term and the combination of antiandrogens and AIs has also been proposed. Caution must be used with the use of ketoconazole given the risk of liver toxicity. Non-classical forms of CAH should be managed with glucocorticoids (Chapter 9).

Delayed Puberty

Definition

DP is one of the commonest reasons for referral to pediatric endocrinologists. Additionally, many of the adolescents consulting for short stature have their growth trajectory influenced by a variation or an anomaly related to pubertal timing. DP is defined by the lack of pubertal development at an age when 97.5% of the general population have reached the relevant milestones. A clear definition is not straightforward given the wide variety of factors associated with pubertal timing and the number of milestones that should be integrated for a thorough evaluation of puberty. In simple terms, DP is generally defined in girls by the lack of breast development at the age of 13 years or by the absence of menarche at the age of 15 years and in boys by the lack of testicular development above 3 mL at the age of 14.0 years.

Differential Diagnosis

The pathogenesis of DP encompasses several conditions but is most commonly due to self-limited DP due to functional hypogonadism, disorders causing primary

Table 7.8 Differential diagnoses of self-limited delayed puberty.

	Hypergonadotropic hypogonadism	Hypogonadotropic hypogonadism	Functional hypogonadotropic hypogonadism
Common causes:	*Male:* Klinefelter syndrome Congenital anorchia/testicular regression Mumps orchitis, Coxsackie virus *Female:* Turner syndrome Premature ovarian insufficiency *Both:* Disorders of sexual development Gonadal dysgenesis Chemotherapy/radiation therapy Galactosaemia	Isolated hypogonadotropic hypogonadism Kallmann syndrome Combined pituitary hormone Deficiency CNS tumours/infiltrative diseases Chemotherapy/radiation therapy	Inflammatory bowel disease Coeliac disease Anorexia nervosa Hypothyroidism Excessive exercise

Source: Table modified and reprinted with permission from Palmert and Dunkel [56].

hypogonadism and GnRH deficiency leading to HH (Table 7.8) but up to 30 different aetiologies underlying DP have been identified [54].

The absence of pathological medical history, signs and symptoms and a positive family history of pubertal delay in one or both of the parents suggests a diagnosis of self-limited DP but, before making the diagnosis, significant pathological conditions must be excluded. These include functional HH, where late pubertal development is due to maturational delay in the HPG axis secondary to chronic disease (found in 19–20%), malnutrition, excessive exercise, psychological or emotional stress, hypergonadotropic hypogonadism, with primary gonadal failure leading to elevated gonadotropin concentrations due to lack of negative feedback (found in ~7% of male patients and 26% of female patients with DP) and permanent HH, characterized by low LH and FSH concentrations (9% of boys and up to 20% of girls [55] (Table 7.8).

Self-Limited Delayed Puberty

Self-limited DP, also known as constitutional delay of growth and puberty (CDGP), is the commonest cause of DP in both sexes. The term 'self-limited' has become preferred because, in the absence of an identifiable underlying cause, pubertal onset usually occurs by the age of 18 years and not all patients with 'simple' DP have constitutional features such as growth delay. 83% of boys and 30% of girls with pubertal delay have self-limited DP [55] and they lie at the extreme end of normal pubertal timing with the absence of testicular enlargement in boys or breast development in girls at an age that is 2–2.5 standard deviations (SD) later than the population mean. Self-limited DP may also encompass older children with delayed pubertal progression, a diagnosis that is aided by the use of puberty nomograms (Figure 7.3).

Self-limited DP is considered by many to be a benign developmental variant with no long-term consequences but patients with self-limited DP are at risk of short adult height, decreased bone mineral density and compromised psychosocial health. Later voice breaking in men has been significantly associated with anxiety disorders, chronic fatigue syndrome, depression, asthma and poor overall health. Late menarche (15–19 years) is associated with increased risk of early menopause, osteoporosis, malabsorption, low intelligence, asthma and poor overall health.

Self-limited DP segregates within families with complex patterns of inheritance including autosomal dominant, autosomal recessive, bilineal and X linked, although sporadic cases are also observed. The majority of families display an autosomal dominant pattern of inheritance (with or without complete penetrance) [57]. 50–75% of subjects with self-limited DP have a family history of delayed pubertal onset [58]. Self-limited DP is not sex specific, as near-equal sex ratios among family members are seen [59]. Although a predominance of males presenting with the condition has been noted, this may be a consequence of referral bias.

The neuroendocrine pathophysiology and its genetic regulation remain unclear in the majority of patients with DP. Only rarely have mutations in genes known to cause aberrations of the HPG axis been identified in cases of self-limited DP although mutations in *IGSF10* have been implicated in the pathogenesis of late puberty in families from a large Finnish cohort with familial DP [60]. Mutations in *IGSF10* appear to cause dysregulation of GnRH neuronal migration during embryonic development, which presents in adolescence as DP without previous constitutional delay in growth. *IGSF10* loss-of-function mutations were also discovered in patients with a hypothalamic amenorrhoea-like phenotype.

Rare heterozygous variants in *FTO* have been identified in pedigrees with self-limited DP associated with extreme low BMI and maturational delay in growth in early childhood [61]. Notably, mice that were heterozygous for *FTO* gene knockout displayed significantly delayed timing of puberty without significant reduction in body mass. There is evidence that mTOR plays a central role in the coupling of energy balance and HPG axis activation via modulation of hypothalamic expression of Kiss1. Blockade of mTOR caused delayed vaginal opening in rodents with blunting of the positive effects of leptin on puberty onset in food-restricted females. It remains to be determined if the effect of *FTO* on pubertal timing in self-limited DP is mediated via effects on body mass, via mTOR signalling, or both.

Congenital Hypogonadism

CHH is defined by the diagnosis of gonadotropic deficiency during the mini-puberty or in adolescence when puberty is absent or arrested. Sometimes CHH may present in adults due to infertility. A picture of 'idiopathic' hypogonadotropic hypogonadism (IHH) with no associated anatomical or functional defect in the HPG axis occurs in 1–10 cases per 100,000 births. Kallmann syndrome (KS) (HH associated with anosmia) is the most common form of isolated HH. Because of different causes and incomplete penetrance, there is a wide spectrum of phenotypes, ranging from complete HH with lack of pubertal development to partial hypogonadism with an arrest of pubertal development and HH, which is reversible in some patients post-treatment [62]. IHH is reported to be between two and five times more common in boys than in girls.

Different mechanisms of CHH are described: (1) defects in GnRH synthesis that mainly result from an abnormal migration of GnRH neurons from the olfactory placode towards the hypothalamus during the first trimester of fetal life, (2) low GnRH secretion due to a defect of GnRH secretagogue bioactivity such as kisspeptin or neurokinin B, (3) poor maturation of the GnRH neuronal network and (4) loss of function of GnRH itself or its receptor also known as a defect of the bioactivity of GnRH [63].

The clinical presentation of CHH is related to the severity of GnRH deficiency and to associated biological features. The severity of the gonadotrophic axis deficiency determines the phenotype at birth and at adolescence. A functional gonadotrophic axis *in utero* is required for the normal development of primary sexual characteristics at least in boys (Section: The Reactivation of the Gonadotropic Axis at Puberty). At birth, a suspicion of hypogonadism may be raised by the assessment of genital appearance. In congenital GnRH deficiency, fetal and postnatal pituitary gonadotropin secretion is low and boys often have micropenis and cryptorchidism at birth. The incidence of CHH in isolated congenital bilateral undescended testes has been reported to be as high as 70%. Although puberty is recognized as the maturational process of the reproductive endocrine system that results in achievement of adult body proportions and reproductive capability, mini-puberty has been increasingly recognized as vital for normal fertility development. Mini-puberty provides a window of opportunity for evaluation of the functionality of the HPG axis before puberty.

During childhood, the gonadotropic axis is dormant and LH is detectable only by ultrasensitive assays; FSH plasma concentrations are variable. The diagnosis of CHH is difficult to establish during this period. CHH is frequently diagnosed in adolescence due to a lack of initiation of puberty. In a few cases, the diagnosis is made in adulthood due to infertility. The diagnosis of CHH in boys at puberty or in adulthood without clinical signs at birth suggests partial gonadotropin deficiency. Due to the absence of a phenotype at birth, this correlation does not apply to females.

CHH may also be suspected by the presence of associated clinical features, which helps to classify CHH into three categories and points towards the underlying pathogenic mechanism. The first group is isolated CHH. The association of CHH with anosmia defines KS as a second group. Hearing impairment and skeletal abnormalities such as ectrodactyly, synkinesia (upper limb mirror movements), cleft lip/palate and hypodontia may also be observed in KS. In addition to these relatively common clinical features, CHH may also be a component of a more complex syndrome (syndromic CHH) (Table 7.9). Obesity, abnormal behaviour, ataxia, mental disability, neuropathy or white matter disorder may be observed in these syndromes. In a few cases, CHH may be associated with a neurodegenerative process starting in adolescence.

Patients with CHARGE syndrome (*c*oloboma, *h*eart malformations, *a*tresia of the choanae, *r*etardation of growth and development, *g*enital anomalies and *e*ar anomalies both auditory and vestibular) may have central hypogonadism and HH may be present because the majority of patients with CHARGE syndrome have olfactory bulb aplasia. CHARGE syndrome has an estimated birth incidence of 1 in 8,500–12,000. Other infrequently occurring features include characteristic face and hand dysmorphia, hypotonia, arhinencephaly, semicircular canal agenesis or hypoplasia, hearing impairment, urinary tract anomalies, orofacial clefting, dysphagia and tracheoesophageal anomalies. Multiple sets of diagnostic criteria for CHARGE syndrome have been proposed [64].

The causative CHD7 gene encodes a chromodomain (chromatin organization modifier domain) helicase DNA-binding protein expressed in the olfactory placode,

Table 7.9 Syndromes associated with hypogonadotropic hypogonadism.

Syndrome	Phenotype	Genetic defect
Prader–Willi	Mental retardation, morbid obesity, hypotonia	Deletions within paternally imprinted 15q 11.2–12 region
Bardet–Biedl	Mental retardation, obesity, retinitis pigmentosa, postaxial polydactyly	BBS-1-11 (multiple loci) 20p12 16q21, 15q22.3–23, 14q32.1
Biemond (brachydactyly–nystagmus–cerebellar ataxia)	Iris coloboma, polydactyly, developmental delay	
CHARGE anomaly (see text)	Coloboma, heart malformations, choanal atresia, growth retardation, genital anomalies and ear anomalies, hypogonadotropic hypogonadism, olfactory bulb aplasia/hypoplasia	*CHD7*
Adrenal hypoplasia congenita	Primary adrenal deficiency	*DAX-1*
Septo-optic dysplasia	Small, dysplastic pale optic discs and other eye defects Pendular nystagmus Midline hypothalamic–pituitary defect with variable DI, GH, ACTH, TSH and LH/FSH deficiencies, absent septum pellucidum and other midline defects	*HESX1, OTX2, TCF7L1, SOX2*
Solitary median maxillary incisor syndrome	Prominent mid-palatal ridge	SHH 7q3, *FOXA2*
Borjeson–Forssman–Lehmann syndrome	Mental retardation	X-linked ataxia
Gorden Holmes	HH, ataxia, dementia	Inactivating mutations in *RNF216/OTUD4* *PNPLA6*
Lawrence Moon	Cerebellar ataxia, ocular defects, intellectual disability, spastic paraplegia, short stature, HH	*PNPLA6*
Oliver–McFarlane	Trichomegaly, severe chorioretinal atrophy and multiple pituitary hormone deficiencies (GH, TSH and gonadotrophins)	*PNPLA6*
4H syndrome	Hypomyelination, hypodontia and HH	*POLR3A/B*
Warburg Micro	Ocular, neurodevelopmental and central reproductive defects	*RAB3GAP, RAB18*

which gives rise to GnRH neurons, spinal cord, nasopharynx and eye. This protein may explain some of the organ involvement. Most patients are heterozygous for loss-of-function mutations in *CHD7* [65].

CHH may be sporadic or familial. It is more frequent in girls than boys. The molecular basis of this sexual dimorphism is not clear and probably reflects the normal sexual dimorphism of the gonadotropic axis function. Familial CHH is rare. It was initially considered a monogenic disorder but several modes of transmission have been described: X-linked recessive transmission, autosomal recessive transmission, autosomal dominant transmission or transmission linked to an imprinting locus (Table 7.10).

- *Recessive transmission*. Isolated CHH is transmitted as a recessive autosomal trait due to mutations in three pairs of neuropeptide receptors. Loss-of-function mutations in *KISS1* and its receptor *KISS1R*, as well as neurokinin B encoded by *TAC3* and its receptor *TACR3*, have been described in informative families by linkage analysis. Mutations in *GnRH1* and its receptor, *GnRHR*, have been characterized by a candidate gene approach.
- *X-linked transmission*. This mode of transmission has been observed only in KS. *ANOS1* is the only KS gene located on the X chromosome reported to date. *ANOS1* mutated boys are affected whereas females are unaffected but carriers.
- *Autosomal dominant transmission*. Very rare variants in several autosomal genes have been reported in the dominant form of KS. For some of these genes, the transmission is caused by a monoallelic pathogenic mutation whereas in others an additional genetic event is awaited to fully explain the phenotype. This group includes syndromes with olfactory bulb agenesis in which CHH is transmitted as a dominant trait such as in CHARGE syndrome.

Table 7.10 Genetic causes of hypogonadotropic hypogonadism and associated features.

Primary/congenital					
	Isolated	**KS**	**Syndromic**	**Pathogenic variants**	**Associated variants**
GnRHR/GNRH1	x			x	
KISS1R/KISS1	x			x	
TACR3/TAC3	x			x	x
FGFR1/FGF8	x	x	Hartsfield	x	x
FGF17	x	x			x
ANOS1 (KAL1)		x		x	
HS6ST1	x	x			x
IL17RD		x			x
DUSP6	x	x			x
SPRY4	x	x			x
FLRT3		x			x
PROKR2/PROK2		x		x	
SEMA3A, SEMA3E/ SEMA7A		x			x
WDR11	x	x		x	
CCDC141	x	x			x
LEPR/LEP			Severe obesity	x	
PCSK1			Obesity, ACTH deficiency, diabetes	x	
DMXL2			Polyendocrinopathy Polyneuropathy syndrome	x	
RNF216/OTUD4		x	Gordon Holmes	x	
PNPLA6		x	Gordon Holmes, Oliver Mcfarnlane, Lawrence Moon	x	
SOX10		x	Wardenburg	x	
FEZF1		x			x
CHD7	x	x	CHARGE	x	
POLR3A/POLR3B			4H	x	
LHB	x			x	
FSHB	x			x	
NROB1			Adrenal hypoplasia	x	
Associated with other pituitary hormone deficiencies					
Congenital	with or without midline defects				
	with or without developmental defects				
Secondary	Tumors: craniopharyngioma, Germinoma, Astrocytoma, glioma				
	Rathke pouch cyst				
	Brain (pituitary) irradiation				
	Head Trauma				
	Infiltrative diseases: Haemochromatosis, Histiocytosis, sarcoidosis				
Functional, secondary to:					
.Chronic diseases: gastrointestinal (coeliac disease, inflammatory bowel disease),					
.Endocrinopathies: hypothyroidism, hyperprolactinemia, GH deficiency, glucocorticoïd excess (Cushing syndrome, iatrogenic)					
.Psychiatric illness: anorexia nervosa					
.Excessive exercice, undernutrition					

- *Syndromic CHH*. The majority of cases of syndromic CHH are transmitted as a recessive autosomal trait, except for *DAX1*, which is X linked. There is one exception: Prader–Willi syndrome is an imprinting disorder due to a defect of the expression of the paternal allele from chromosome 11.

Although CHH cases are mainly sporadic, a detailed analysis of the pedigree may be informative when making the diagnosis. Recessive transmission is suspected when parents are consanguineous or when the phenotype is observed in two siblings but parents are unaffected. In those cases, rare variants are present in the paternal and maternal allele of candidate genes. An identical variant in both alleles suggests that parents are consanguineous but heterozygote composite variants are more frequent than homozygous variants in isolated CHH. The most frequent pathogenic variant in *GnRHR* (p.Gln106Arg) causes only a partial inactivation of the receptor activity, which explains the relatively high frequency of this variant in the general population, with a minor allele frequency of 0.3%. X-linked transmission is suspected when the mother is unaffected and one related male is affected. Anosmia is frequently observed in X-linked CHH and sequencing of *ANOS1* for rare variants is then advised to confirm the diagnosis.

Dominant transmission of CHH is more frequent in KS than in isolated CHH. This mode of transmission is relatively surprising for a reproductive disorder causing infertility in adulthood. Such mutations may give rise to a partial phenotype or one with variable severity of gonadotropin deficiency. The familial transmission of developmental features such as anosmia and cleft lip/palate may be taken into account to determine the dominant mode of transmission. In the same family, isolated gonadotropic deficiency, KS or isolated anosmia may be observed. The molecular genetics of KS has been much improved by the screening of large cohorts of patients using NGS techniques. These studies have led to the definition of two groups of genes with monoallelic variants: the first consists of genes in which rare monoallelic pathogenic variants have been confirmed by several independent studies; the second comprises genes in which monoallelic variants of unknown significance are more frequent in the patient group as compared with the control population [65].

For the majority of familial KS, the molecular diagnosis is obvious as the observed variant is pathogenic. Associated clinical features such as an ectrodactyly may be highly predictive of a pathogenic variant [66]. For another group of patients, it is not possible to affirm the link between genotype and phenotype. The incomplete penetrance observed for these variants of unknown significance has led investigators to propose an oligogenic model of transmission. In this model, the probability of developing and also the severity of CHH are due to the association of several rare variants in candidate genes. The frequency of this oligogenic transmission remains unknown. The fact that age at menarche and at puberty are polygenic traits with hundreds of loci involved strongly supports the hypothesis that some CHH cases could indeed be transmitted as an oligogenic trait or even could be considered as part of a polygenic disorder. The fact that CHH may be reversible in adulthood in a significant proportion of patients is probably related to this polygenic effect. A brief discontinuation of treatment to assess the reversibility of gonadotropin deficiency must be discussed with patients [67].

GnRH Resistance and Gonadotropin Deficiency

Loss-of-function mutations within the GnRH receptor are the most frequent cause of autosomal recessive IHH, accounting for 16–40% of patients. Mutations have been found within the extracellular, transmembrane and intracellular domains of the receptor leading to impaired GnRH action [68]. Treatment is with gonadotropins.

LH and FSH are glycoprotein hormones encoded by a common α-subunit gene and a specific β-subunit gene. Mutations of the β-subunits of LH or FSH are rare causes of HH [30]. Females with inactivating mutations of *LHβ* present with onset of normal puberty and normal or late menarche followed by infertility due to lack of ovulation. Males with inactivating mutations of the *LHβ*-subunit have absent pubertal development with Leydig cell hypoplasia leading to testosterone deficiency and azoospermia. Individuals with inactivating *FSHβ* mutations present with incomplete pubertal development and primary amenorrhoea in females and azoospermia in males.

Functional HH

Chronic Disease

A wide variety of childhood diseases including many with chronic inflammation such as Crohn's disease, coeliac disease, chronic kidney disease, cystic fibrosis, sickle cell disease and juvenile idiopathic arthritis are associated with an increased likelihood of DP. This is a result of several factors related to the disease itself, such as malnutrition, hypercortisolaemia and elevated levels of pro-inflammatory cytokines. In malnutrition and chronic diseases, weight loss of <80% of ideal body weight can cause delayed or arrested pubertal development. Nutrition plays an important yet uncharacterized role in the control of GnRH secretion. For example, in regional enteritis, gonadotropin secretion remains normal if nutrition is adequate but a poor nutritional status will result in a hypogonadotropic state and arrested pubertal maturation. Poor nutrition also contributes to the decrease in growth velocity, decreased bone mineral

density and low mood that are often observed in these patients. Chronic renal insufficiency delays pubertal development, but gonadotropin secretion is usually restored after successful renal transplantation.

Most endocrinopathies can cause functional HH with DP, arrested puberty or functional amenorrhoea [69]. The pathophysiology is mediated by hyperprolactinaemia itself or via interference with the inhibitory effect of dopamine on prolactin secretion, by the suppression of GnRH, by excess cortisol and/or by hyperandrogenaemia. Treating the underlying endocrinopathy usually results in normalization of the HH axis.

Anorexia

Anorexia nervosa is associated with severe or even fatal weight loss due to distorted body image, obsessive fear of obesity and avoidance of food. Virtually all patients have primary or secondary amenorrhoea. Functional HH is due partly to severe weight loss but amenorrhoea may precede its onset. The underlying pathophysiology of amenorrhoea is due to GnRH deficiency because the LH secretory pattern in pubertal-aged girls with anorexia is similar to that seen in girls during prepuberty with low or absent LH pulses and blunted LH response to exogenous GnRH. Long-term pulsatile administration of GnRH has been shown to restore a pubertal pattern of LH secretion, confirming the hypothalamic location of the defect. Recovery of normal weight will normalize most endocrine and metabolic functions but amenorrhoea may persist for years.

Athletic Training

Excessive intensive exercise may suppress the HPG axis by inhibiting hypothalamic pulsatile secretion of GnRH, arrest pubertal development and cause amenorrhoea in females. This includes compulsive endurance training and is common among long-distance runners, gymnasts and ballerinas. HH may develop when athletes have normal weight but have less fat and more muscle compared with non-athletic individuals. In female athletes with delayed or arrested pubertal development, adrenarche usually occurs at the normal age. The mechanism of the DP is unclear but interruption of intensive training advances puberty and menarche before any change in body composition or weight, suggesting a direct effect of physical activity on GnRH secretion.

Central Nervous System Tumours

Tumours causing DP interfere with GnRH synthesis or secretion [62]. Deficiency of other pituitary hormones is common. Associated posterior pituitary hormone deficiencies are often manifested by diabetes insipidus (DI).

Craniopharyngioma

The most common neoplasm causing hypothalamic–pituitary dysfunction and hypogonadism is craniopharyngioma, a congenital tumour that most commonly becomes symptomatic between 6 and 14 years of age. At presentation, the most common symptoms are headache, visual disturbances, short stature, DP, polyuria and polydipsia. The structure of the tumour varies from solid to cystic. MRI with and without contrast enhancement is the imaging modality of choice. While the characteristic calcifications and size of the tumour can be visualized with CT, the size and extent of the tumour, involvement of the third ventricle and cystic features of the tumour can be confirmed with MRI. Treatment consists of surgery and radiotherapy but the recurrence rate is high even when complete surgical removal is attempted (Chapter 5).

Langerhans Cell Histiocytosis

Langerhans cell histiocytosis (LCH), also called Hans–Schüller–Christian disease, Abt–Letterer–Siwe disease or histiocytosis X, is characterized by infiltration of lipid-containing histiocytic cells in the skin, bone, and viscera. CNS and hypothalamic–pituitary involvement are well-described features. The incidence of CNS–LCH disease is unknown and the natural history poorly understood. DI is reported to be the most common and well-described manifestation of hypothalamic–pituitary involvement in up to 50% of cases. Anterior pituitary dysfunction has been reported in up to 20% of patients and occurs almost exclusively concurrently with DI. The natural course of the disease is fluctuating, making evaluation of treatment effect difficult. Endocrine function does not improve following medical treatment of LCH with chemotherapy and glucocorticoids. All LCH patients should undergo a thorough endocrine evaluation periodically.

Germinomas

Germinomas are the most common extrasellar tumours to cause DP, although these tumours are a rarity among primary CNS tumours. Polydipsia, polyuria, and visual disturbance are the most common symptoms followed by arrested growth and DP. Germinomas are commonly located in the pituitary stalk, in the suprasellar region of the hypothalamus or near the pineal gland. Seeding of the tumour to the cerebrospinal fluid is common and can be used in the diagnosis by examination of tumour markers (βhCG and alpha-fetoprotein) or germ cells (with positive placental alkaline phosphatase staining) in the cerebrospinal fluid. These laboratory findings, together with clinical features and an excellent response to radiation therapy, are so characteristic that surgery is rarely indicated except for biopsy to establish the histological diagnosis.

Treatment of CNS tumours, leukaemia or neoplasms with cranial irradiation may result in gradual development of hypothalamic–pituitary failure. GH deficiency is the most common component of the radiation-induced hormone disorder but gonadotropin deficiency also occurs when the radiation dose is high enough. Development of radiation-induced hypothalamic–pituitary failure usually takes from 1 year to several years to ensue.

Developmental Defects of the Central Nervous System

Various malformations affecting the development of the prosencephalon may cause DP combined with deficiency of any or all other pituitary hormones. Midline malformations are often associated with optic nerve hypoplasia and an absent septum pellucidum is often found by imaging techniques (septo-optic dysplasia). Other congenital midline defects ranging from holoprosencephaly to cleft lip and palate may also be associated with variable hypothalamic–pituitary dysfunction (see Chapter 5).

Genetic defects affecting development of the anterior pituitary cause hypopituitarism, including HH, in some cases. The pituitary transcription factors, *HESX*1, *LHX3* and *SOX2*, are vital for early patterning of the forebrain and pituitary and mutations in these developmental genes result in syndromic hypopituitarism with gonadotropin deficiency in humans. *PROP1* is important for the development of gonadotropin-secreting cells and mutations in this gene are the most common cause of combined pituitary hormone deficiency in humans. *PITX2* is also vital for survival of gonadotroph cell lineage and is required for expression of the gonadotroph-specific transcription factors *GATA2*, *EGR1* and *SF1*.

The *DAX-1 orphan nuclear receptor gene (DAX1)* and *steroidogenic factor-1 (NR5A1, SF1)* are important for the development of the adrenal gland, gonads, ventromedial hypothalamus and pituitary gonadotroph cells. Mutations in *DAX1* cause X-linked adrenal hypoplasia congenita, with associated HH, while mutations in *SF1* are associated with 46XY sex reversal or gonadal dysgenesis and 46XX premature ovarian insufficiency (POI). Leptin and prohormone convertase-1 may also influence GnRH release and processing of the GnRH receptor, with mutations resulting in a phenotype of HH.

Hypergonadotropic Hypogonadism

Conditions associated with primary gonadal failure are listed in Table 7.8. Elevated serum gonadotropins occur usually by the time of the physiological age of puberty. During mid-childhood, serum gonadotropins may be similar or mildly higher than those in normal controls. In boys, low serum inhibin B reflects primary germ cell failure.

Chromosomal Abnormality

Klinefelter syndrome in boys and Turner syndrome in girls are the commonest chromosomal abnormalities seen with hypergonadotropic hypogonadism. Both syndromes are caused by an abnormal number or structure of the X chromosome with a predisposition towards autoimmune diseases, metabolic and cardiovascular disorders and certain cancers.

Klinefelter syndrome (46,XXY) has an incidence of ~1 in 800. Onset of puberty is usually not delayed but arrest of puberty is almost uniformly observed and in non-mosaic forms testicular volume rarely exceeds 6 mL. Most patients are identified after puberty with small testes and infertility [70]. Varying phenotypes are described, with gynaecomastia due to increased oestrogen-to-testosterone ratios, disproportionately long limbs, tall stature, poor muscular development and potential learning and psychosocial problems [71].

Turner syndrome, with partial or total (45,X) absence of one of the X chromosomes or a structurally abnormal X chromosome such as an isochromosome or ring chromosome, occurs with an incidence of about 1/2500 live-born females. About half of girls with Turner syndrome have the 45,X karyotype but 99% of fetuses with this karyotype abort spontaneously, and the fetus has the 45,X karyotype in 1 of 15 spontaneous abortions. Chromosomal mosaicism and structural abnormalities of the sex chromosomes modify the clinical features. The condition may be identified during the prenatal or neonatal period incidentally by features such as lymphoedema, pterygium colli and coarctation or diagnosed in adolescence because of growth and pubertal failure or in adult life because of primary or secondary amenorrhoea and infertility [72].

Typical features include short stature, which may be apparent already at birth, lymphoedema and loose posterior cervical skinfolds during the newborn period, low-set or deformed ears, epicanthal folds, ptosis, micrognathia, high arched palate, dental abnormalities, wide-spaced nipples caused by shield-like chest, hypoplastic areolae, short neck with low hairline and cubitus valgus. Abnormalities of the left side of the heart include coarctation of the aorta, aortic stenosis and bicuspid aortic valves. Renal anomalies include abnormal position or alignment (horseshoe kidney) and various anomalies of the collecting system. Patients with Turner syndrome have an increased incidence of inflammatory bowel disease, autoimmune thyroiditis, Graves' disease and insulin resistance. Intelligence is usually normal but spatio-temporal processing, visuomotor coordination and mathematical skills may be impaired. The greatest cause of reduced life expectancy is dissection or rupture of the aorta, with risk factors that include hypertension, a bicuspid

aortic valve and dilatation of the root of the aorta. Cardiovascular complications represent a major concern during pregnancies in these women, which usually need to be medically assisted.

Ovarian insufficiency is also apparent at birth, as evidenced by high gonadotropin concentrations during the neonatal period. During childhood, with the development of the CNS-mediated inhibition of GnRH secretion, gonadotropin concentrations decrease to near-normal concentrations, but they are usually elevated again by 10 years of age. The Müllerian structures (uterus and fallopian tubes) are present but remain infantile if the ovarian failure is not adequately treated with hormone replacement therapy. Histologically, the ovaries are streaks of connective tissue, with a decreased number of primordial follicles and oocytes for age. The condition may be associated with gonadoblastoma if a Y chromosome is present in the genome. Spontaneous oocyte death is accelerated, resulting in premature loss of the oocyte pool.

Sexual infantilism is one of the most common clinical findings in girls with Turner syndrome. More than 90% have gonadal failure but up to 30% of girls undergo some spontaneous pubertal development and 2–5% have spontaneous menses and may have the potential to achieve pregnancy without medical intervention. Pubertal development may be delayed and is followed by progressive ovarian failure in most patients [72].

Most patients are small-for-gestational age at birth and a slow growth rate is apparent after 3 years of age. Short stature results partly from the haploinsufficiency of the SHOX gene on the distal part of the short arm of chromosome X. Most girls fail to have a pubertal growth spurt due to insufficient oestrogen production in the ovaries. The mean adult height is 143–146 cm if untreated, ~20 cm lower than that of unaffected women, depending on both parental heights and the overall height of the same genetic population [73].

Several complex syndromes are associated with hypergonadotropic hypogonadism and include Down syndrome, hypogonadism associated with myopathies (myotonic dystrophy and progressive muscular dystrophy) and Werner and Alström syndromes. In Noonan syndrome and related disorders, testicular abnormalities are less severe.

46 XX and 46 XY Gonadal Dysgenesis

The term 'pure gonadal dysgenesis' refers to phenotypic females with no pubertal development and a 46,XX or 46,XY karyotype without detectable chromosomal abnormalities. Patients with 46,XX gonadal dysgenesis have normal stature, bilateral streak gonads, normal female internal and external genitalia and (sometimes) sensorineural deafness. Malignant transformation of the streak gonad is rare. Most cases are sporadic but an autosomal recessive form has been described; few causal genes have been identified in these patients. Patients with familial or sporadic 46,XY gonadal dysgenesis have normal female internal and external genitalia with occasional clitoral enlargement due to increased testosterone production by the gonad, bilateral streak gonads and normal or tall stature with eunuchoid body proportions. The dysgenetic gonads may undergo neoplastic transformation, so gonadectomy may be indicated. XX gonadal dysgenesis can occur in combination with cerebellar ataxia and learning difficulties or with multiple malformation syndromes with a range of associated features including microcephaly, limb abnormalities and facial and cardiac defects.

Premature Ovarian Insufficiency

The term POI describes a continuum of declining ovarian function and comprises a heterogeneous spectrum of disorders from ovarian follicular dysfunction to ovarian dysgenesis [74]. Genetic causes are identified in about 20% of cases but in most cases the aetiology is unknown. Family history is reported in 10–15% of cases. POI may be isolated or syndromic, such as in Turner syndrome, blepharophimosis syndrome, galactosaemia, which is caused by a deficiency of galactose-1-phosphate uridyltransferase (GALT) enzyme activity, and autoimmune polyendocrinopathy.

The main genetic causes identified to date are chromosomal abnormalities (Turner syndrome, various microdeletions on the short and long arms of the X chromosome, X-autosome translocations) and mutations in several genes including *POF1*, *POF2*, *DIAPH2*, *FMR1*, *FOXL2*, *BMP15*, *GDF9*, *NOBOX*, *NR5A1*, *CPEB1* and most recently *STAG3* [75]. In adolescence, POI is often recognized by menstrual cycle irregularity or amenorrhoea. High serum concentrations of gonadotropins with low serum oestradiol and AMH concentrations confirm the diagnosis.

Gonadotropin Receptor Mutations

Several homozygous or compound heterozygous loss-of-function mutations in the *LHCGR* gene have been described in males and females. The presentation in females is usually with primary amenorrhoea rather than DP. In XY males, the lack of virilization during fetal development results in a female phenotype with the absence of Müllerian structures and the absence of Leydig cells in the testis. Serum concentrations of LH are elevated and FSH concentrations are normal. Homozygous mutations in the *follicle-stimulating hormone receptor* (*FSHR*) are extremely rare, affecting mostly females with variable degrees of pubertal development and complete ovarian failure. Discovered first in

the Finnish population, point mutations in the extracellular domain of the *FSHR* lead to subsequent inactivation of receptor function resulting in raised FSH concentrations [76]. While up to 40% of Finnish patients with POI have such a mutation, these appear to be rare in other populations.

Histological examinations of ovarian biopsies show the presence of follicles in all female patients with FSH receptor defects, whereas only one in four of those with unknown aetiology has follicles. Hence, whereas the receptor defect causes a specific arrest in follicular maturation, many patients with hypergonadotropic ovarian failure have true ovarian dysgenesis. The ovarian phenotype in patients with inactivating FSHR mutations is informative with regard to the role of FSH in the regulation of follicular development: the early phases of follicular maturation (up to the pre-antral stage) are independent of FSH but this gonadotropin is absolutely necessary for the final maturation of the follicle. In males, serum LH and testosterone concentrations are normal and FSH concentrations are elevated.

Autoimmune Ovarian Insufficiency

Autoimmune ovarian insufficiency is often one of the components of autoimmune polyendocrinopathies. Autoimmune polyglandular syndrome type I is an autosomal recessive disorder caused by mutation in the *autoimmune regulator* (*AIRE*) gene, which maps to 21q22.3. It is characterized by two of the three major clinical symptoms that may be present: hypoparathyroidism, Addison's disease and chronic mucocutaneous candidiasis. Candidiasis is the initial manifestation in 60% of the patients and is present in all patients at some time, often preceding symptoms and signs of endocrinopathy [77]. Hypoparathyroidism usually reveals itself before adrenal insufficiency. Additional features include hypoplasia of the dental enamel and keratopathy not attributable to hypoparathyroidism. Some of the manifestations of the disorder do not appear until the fifth decade. Thus, all patients need lifelong follow-up for the detection of new components of the disease. Gonadal failure is seen in 60% of female patients over 13 years of age. Other autoimmune diseases associated with primary ovarian insufficiency include myasthenia gravis, systemic lupus erythematosus and rheumatoid arthritis (Chapter 11).

Other causes of Male Gonadal Failure

Bilateral anorchia is defined as the absence of testicular structures in a 46 XY phenotypically male subject. Absence of the testes can be diagnosed either in the neonatal period or later in life. The prevalence remains unknown and is estimated to occur in about 1 in 20,000 males. Micropenis is associated with bilateral anorchia in almost half of the cases. To explain the vanishing testis syndrome, various causes have been proposed including bilateral testicular torsion and/or genetic predisposition. The mechanism remains unknown. Infective causes include mumps orchitis, now rare due to the childhood vaccination programme, and Coxsackie virus.

Disorders of Sex Development

Many causes of DSD are associated with gonadal failure during fetal development (see Chapter 4).

Oestrogen Receptor Mutations

Homozygous loss-of-function mutations in *ESR1* have been described as a rare cause of severe oestrogen resistance in women who have absent breast development and markedly elevated serum concentrations of oestrogens and bilateral multicystic ovaries.

After Treatment for Childhood Cancer

Childhood cancer survivors (CCS) are at increased risk of primary gonadal insufficiency. Gonadal failure may affect all aspects of reproductive health, including pubertal development, hormone production, sexual function and fertility. The degree of risk of endocrine deficiency is related to the child's sex and their age at the time the tumour was diagnosed, as well as to tumour location and characteristics, and the nature of therapies used, including surgery, total dose and nature of gonadotoxic chemotherapy such as alkylating agents, radiation dose and fractionation schedule. Most chemotherapy protocols use multiple agents, the effects of which may be synergistic.

Abdominal, pelvic and total body irradiation may result in ovarian and uterine damage. The human oocyte is sensitive to radiation, with an estimated LD50 of <4 Gy. Less than 2% of children receiving total body irradiation subsequently become pregnant, although there may be some protection of ovarian function in prepubertal girls. Fertility preservation must be considered where possible before initiation of gonadotoxic treatment but suppression of the pituitary–gonadal axis with gonadal steroids or GnRH agonists does not protect the ovary from the damage induced by irradiation or chemotherapy. Biochemical detection of gonadal damage is rarely possible before puberty, so treatment-induced gonadal damage during childhood if not monitored may present with infertility or premature menopause during adulthood. Surveillance of the onset and pace of puberty is needed in order to start sex steroid treatment as soon as is required.

Pursuit of fertility evaluations after puberty should be proposed in both sexes. Evaluation of the quality and cryopreservation of semen should be proposed in pubertal men. Uterine radiation increases the incidence of nulliparity, fetal loss and small-for-dates infants and it

reduces the success of assisted reproduction. AMH has been shown to be a clear marker of damage to the ovarian reserve of girls receiving chemotherapy for cancer. Ovarian failure may be reversible, more commonly after treatment modalities which do not include radiation therapy (e.g. treatment for osteosarcoma).

Assessment

Complete Clinical Evaluation

A temporary delay in sexual maturation is not uncommon and may resolve with time leading to normal development, optimum final height and fertility but in patients with an underlying organic pathology, early diagnosis and treatment are essential to ensure normal pubertal progress and adequate final height. A medical history must be taken in those presenting with DP, including height and weight charts, nutritional status, medications, history and/or symptoms of chronic disease and psychosocial functioning. Evidence of anorexia and the intensity of athletic training should be noted (Figure 7.15). A history of chronic illness, such as coeliac and inflammatory bowel diseases, may suggest a temporary or secondary delay of puberty. A family history including childhood growth patterns, age at pubertal onset of both parents and siblings and a history of infertility, anosmia and midline abnormalities of parents and siblings is required, as a positive familial history is common.

Physical examination must include pubertal stage assessment in both sexes with penile size and location, as well as size and consistency of the testes. Tanner determination can help to identify early signs of puberty that have not been noticed before. Children who have a low weight for height have an increased likelihood of having an underlying condition delaying HPG axis activation. Bilateral cryptorchidism or a small penis at birth and hyposmia or anosmia due to hypoplasia of the olfactory bulbs may suggest HH. Lack of smell associated with KS can be assessed by means of detailed questioning or objectively by formal olfactory testing, such as the

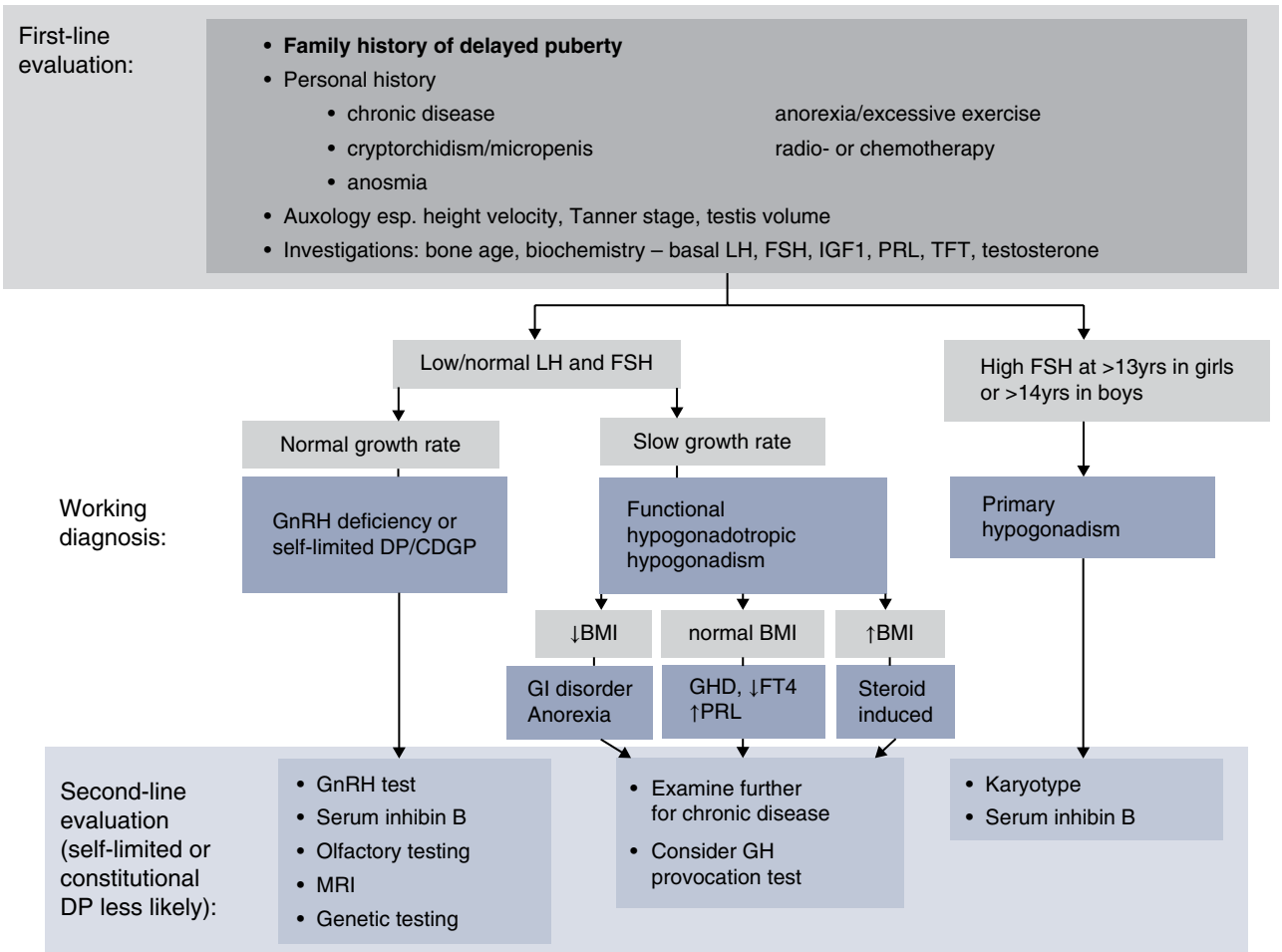

Figure 7.15 Flow chart for the evaluation of a patient with delayed puberty. CDGP, constitutional delay of growth and puberty. *Source:* From Palmert et al. [56], reproduced with permission.

Pennsylvania smell test. Other physical signs will increase suspicion of underlying CHH, such as cleft lip or palate, bimanual synkinesia, congenital ptosis and abnormal visuospatial attention, eye movement abnormalities, sensorineural hearing impairment, agenesis of one or several teeth (hypodontia), obesity and features suggesting the CHARGE syndrome, as well as digital and other skeletal abnormalities. Delayed cognitive development associated with obesity or dysmorphic features may suggest an underlying genetic syndrome. A history of chemotherapy or radiotherapy may indicate primary gonadal failure.

In congenital IHH the diagnosis is typically made during the second or third decade of life. Common presenting signs are delayed onset of puberty, poorly developed secondary sexual characteristics, eunuchoid body proportions or infertility [62]. In some cases, the diagnosis can be suspected before the age of puberty onset during the mini-puberty. The presence or absence of 'red flag' features remains the strongest discriminator between isolated DP and IHH. They include cryptorchidism or micropenis, indicating a lack of prior 'mini-puberty,' and the presence of the other components of KS (e.g. anosmia, cleft lip and palate, unilateral renal agenesis).

Differential diagnosis between self-limited DP and HH in boys who present with DP is often difficult because both conditions may present with the same clinical and hormonal features. Only the demonstration of complete puberty can distinguish isolated DP from HH (partial or complete). Analysis of growth velocity is important in the assessment of individuals with DP; in most subjects with constitutional delay, there is delayed maturation during early childhood and they are short. DP subjects who have poor growth in childhood may not fully exploit their genetic height potential, resulting in an adult height below their mid-parental target height, with an average loss of 4.2 cm if untreated although other studies show a negligible difference in final height, even in DP subjects who have received no intervention. This may imply a pathophysiological mechanism additional to lack of sex steroids contributing to the growth phenotype in some patients with DP but not in others.

Patients with congenital IHH have steady linear growth during childhood and become short for their age only with the absence of the pubertal growth spurt. Hypogonadotropic states cannot be ruled out by short stature and slow growth rate. Adrenarche may occur later than usual in DP compared to the normal age of adrenarche in patients with isolated HH. Bone age in DP is retarded compared with chronological age but developmental milestones are achieved at a normal bone age, i.e. signs of pubertal development by the bone age of 13 years in girls and 13.5 years in boys. While bone age delay provides useful information in the growth analysis, it contributes little to the differential diagnosis. Gonadotropin and testosterone concentrations increase in concert with the development of the bone age. Thus, all stages of pubertal development occur at a later age than usual.

Initial screening in DP should include bone age, completed in relation to clinical features to rule out underlying disorders, basal LH and FSH (to look for hypergonadotropic hypogonadism) and biochemical analysis to search for asymptomatic systemic illness (full blood count, erythrocyte sedimentation rate (or C-reactive protein), renal function, coeliac screen, liver function, electrolytes), thyroid function test, IGF-I and prolactin. A karyotype is important in females with primary hypogonadism. Brain MRI (to examine olfactory bulbs and sulci) is used to exclude olfactory aplasia or hypoplasia or other hypothalamic–pituitary lesions.

Gonadotropin concentrations assessed by basal LH and FSH determination are often increased in females in primary ovarian insufficiency or in Turner syndrome and in males with primary hypogonadism due to, e.g. Klinefelter syndrome but basal gonadotropin values are not useful in the differential diagnosis of self-limited delay from HH. Investigation of the differential diagnosis of these latter two conditions may involve a number of physiological and stimulation tests, including assessment of LH pulsatility by frequent sampling, prolactin response to provocation, gonadotropin response to GnRH, testosterone response to hCG and first morning voided urine FSH and LH [78].

A single measurement of inhibin B of <35 pg/mL in prepubertal boys has been shown to discriminate IHH from CDGP with high sensitivity [79] but this has not been demonstrated in girls. The trio of testicular volume (cut-off 1.1 mL), GnRH-induced maximal LH (cut-off 4.3 IU/L) and basal inhibin B concentration has been proposed as the most effective discriminators of IHH from DP in a recent study [54]. Follow-up is often warranted before a definitive diagnosis can be made.

Renal ultrasound is useful in X-linked CHH due to suspected *KAL1* mutations as it can reveal associated renal malformation or unilateral agenesis. Testicular ultrasound can be useful to identify impalpable gonads and investigate maldescent and their position. Pelvic ultrasound, either transabdominal or, where possible, transvaginal, is useful for female reproductive tract imaging to assess uterine and ovarian size, developing follicles and endometrial thickness.

If gynaecomastia is present in males, examination is important to distinguish true gynaecomastia from pseudogynaecomastia and pathological gynaecomastia. In true gynaecomastia, with the patient lying supine with his hands clasped beneath his head, the breast tissue is

located concentrically under the nipple-areolar complex, feels rubbery or firm and is often bilateral. During the early phase, ductal and epithelial proliferation occurs, which can be tender. Lipomastia or pseudogynaecomastia is characterized by breast fullness and the absence of a nipple-areolar complex mound. Lipomas and neurofibromas are extremely rare in adolescents. The tissue mound in breast cancer feels firm or hard and is located outside the nipple-areolar complex. Nipple discharge, skin dimpling and nipple retraction do not occur in physiologic gynaecomastia.

Assessment of the Newborn

Boys with CHH may present with micropenis and/or cryptorchidism at birth. Primary hypogonadism may also present at birth with underdeveloped genitalia in male infants if the condition is gonadotropin dependent or alternatively as ambiguous or female genitalia if the defect is of early fetal onset resulting in DSD (see Chapter 4).

If a suspicion of congenital hypogonadism arises in the first 3–6 months of life, it can be investigated on the basis of sex steroid and gonadotropin concentrations without the need for stimulation tests. Gonadotropin concentrations in healthy infants start to increase during the first week of life and then decrease towards the age of 6 months, except for FSH concentrations in girls that remain elevated until 3–4 years of age (Table 7.11). Testosterone concentrations in boys increase in response to LH concentrations and peak at 1–3 months of age but in girls oestradiol concentrations fluctuate, probably reflecting ovarian follicular growth and atrophy. Oestradiol concentrations in girls decline in the second year of life. Postnatal HPG axis activation during the mini-puberty has important roles in both sexes, in males for penile and testicular growth and in girls for maturation of ovarian follicles and an increase in oestradiol concentrations.

Most studies on hormone concentrations during mini-puberty have had cross-sectional design and hence the inter-individual differences in timing, duration and magnitude of the mini-puberty have remained largely unexplored. Serial blood sampling from healthy infants is problematic because of its invasiveness, and non-invasive urine or salivary sampling is a way around this problem but urine and saliva assays are not widely used. Longitudinal data have provided new information about the hormonal patterns including the timing of the peak hormone concentrations and the decrease in hormonal activity according to developmental age.

In primary hypogonadism with gonadal dysgenesis, anorchia or testicular regression, gonadotropin concentrations in the mini-puberty are generally raised but may fall to normal concentrations in later childhood (Table 7.11). In Turner syndrome, infant girls with the 45,X karyotype have higher FSH concentrations than healthy girls and the concentrations remain elevated for several years. Turner girls with karyotypes other than 45,X often have close to normal FSH concentrations, suggesting some ovarian feedback effects on pituitary FSH secretion in these patients. Infant boys with Klinefelter syndrome (47,XXY karyotype) often have normal concentrations of inhibin B, AMH and INSL3 suggesting normal Sertoli and Leydig cell function in infancy, although they have elevated LH and FSH concentrations. Testosterone concentrations in these boys are either normal or slightly elevated.

Table 7.11 Upper limits for creatinine-corrected urinary gonadotropin concentrations before term, at term, and at 2–6 months of corrected age.

	Before term	At term	At 2–6 months corrected age
Girls			
FSH (IU/mmol Cr)	250	13	5
LH (IU/mmol Cr)	500	10	0.5
Boys			
FSH (IU/mmol Cr)	10	3	1.5
LH (IU/mmol Cr)	20	5	0.5

Source: Values are based on original data published in Kuiri-Hanninen et al. [80, 81].
Values above the limits suggest primary gonadal failure.

Newer markers of gonadal function are useful, particularly in males, for diagnosis of hypogonadism soon after birth and after the mini-puberty is completed. Inhibin B is a marker of Sertoli cell function from the neonatal period into early childhood and can be used to assess male infants with micropenis and/or cryptorchidism, both due to central and primary hypogonadism. Its use in female infants is less clear. AMH is strongly expressed by Sertoli cells from the time of testicular differentiation to puberty and at much lower concentrations in females by the granulosa cells from birth until menopause. Undetectable AMH and inhibin B are considered diagnostic of anorchia, but low or close to undetectable concentrations are also seen in severe forms of CHH. In infant girls, a similar pattern in AMH concentrations during the first months of life has been reported but the concentrations in girls are significantly lower.

Thus, low sex steroid and gonadotropin concentrations in an infant <3–6 months of age indicate central hypogonadism with an absence of the normal 'mini-puberty' [37].

In contrast, high gonadotropins associated with low/undetectable basal testosterone and INSL3 (in boys) are diagnostic of primary hypogonadism [82]. Outside the mini-puberty period, useful tests for the investigation of hypogonadism include inhibin B and AMH [83].

Management

Most adolescents with DP achieve an adult height in accordance with their genetic height potential and parental target height and most subjects with self-limited DP do not require treatment. A management strategy of 'watchful waiting' may be appropriate in isolated DP, where pubertal onset is late but expected to occur spontaneously. This decision should be taken in conjunction with the patient, taking into consideration their concerns and expectations. Induction or progression of puberty is commonly considered for adolescents who either have significantly delayed or arrested puberty or have been diagnosed with hypogonadism. Therapy may have beneficial effects on body composition and bone mass accretion in addition to the psychological benefits. Appropriate treatment modalities are directed according to the underlying diagnosis.

Adolescents with pubertal delay are usually concerned because of their short stature and lack of pubertal changes in comparison with their peers, leading to psychological suffering and significant anxiety for patients and their parents. The major concern often raised by patients and their families is the effect of DP on both current and adult height. Patients with DP are often short compared with their peers and this is often compounded by the fact that many of the patients who present have pubertal delay combined with familial short stature. Reassurance can be given to such patients as an adult height only slightly below the genetic height potential (target height) is usually reached in DP, although there may be large individual variation. If height is not a major concern, reassurance with accurate adult height prediction is frequently sufficient, especially if puberty has already started.

The effect of DP on body composition with the risk of decrease in bone mineral density and muscle mass as well as on psychological development, behaviour and mood including depression and anxiety should be anticipated and managed. DP in adolescents can be associated with significant anxiety about body image in terms of physical size and pubertal immaturity, decreased self-esteem with social isolation, withdrawal from sporting activities and psychosocial and peer relationship difficulties. Psychological distress may affect behaviour and school performance, decrease self-esteem and persist into young adult life with concern in terms of sexual and reproductive capacities. Without intervention, most subjects undergo spontaneous normal pubertal development and reach their target height. Development may, however, occur several years after that of their peers and many adolescents suffer emotional distress. In these circumstances, there is evidence that hormonal therapy can be beneficial. The link between DP and reduced academic performance, substance misuse and behavioural difficulties is less well established.

Diagnostic difficulty may arise in women with primary amenorrhoea, normal olfaction and no identified mutation where the differential diagnosis lies between CHH and functional hypothalamic amenorrhoea. In such cases, it is important to exclude other causes of HH such as those due to low body mass, eating disorders, excessive physical activity and chronic underlying conditions. Sometimes only after a period of observation with later reassessment of the hypothalamic–pituitary ovarian axis can the diagnosis be established. It is recognized that a genetic lesion responsible for CHH may be present in a significant number of women presenting with hypothalamic amenorrhoea [84].

If 'red flag' markers of hypogonadism are present or endogenous gonadotropin-dependent puberty has not started after one year of treatment, permanent HH and other diagnoses should be considered. In such instances treatment should be initiated promptly in order to optimize skeletal growth and to induce secondary sexual characteristics and, therefore, minimize the psychosocial difficulties. The aim of treatment is to induce the progressive development of secondary sexual characteristics, the pubertal growth spurt, the development of body composition with adequate muscle and adipose tissue growth and repartition, a normal bone mineral density acquisition and the psychosocial well-being. The potential for fertility has to be considered in relation to the aetiology. Support groups and internet resources can provide great benefit for affected individuals and their families. In case of permanent failure, the importance of continuity of treatment into adulthood, communications between specialists and primary care physicians and paediatric and adult endocrinologists must be empathized.

Males

Induction or progression of puberty is commonly considered for adolescents who have significantly delayed or arrested puberty or hypogonadism [85]. Appropriate treatment modalities are directed according to the underlying diagnosis.

Self-Limited DP Management

The options for management of male patients with DP include monitoring with reassurance or therapy with low-dose testosterone to augment growth rate and to induce secondary sexual characteristics (Table 7.12). There are a great number of published studies of treatment of

DP in boys that are mainly observational with some small, randomized controlled trials. Most report treatment with short courses of low-dose androgens with outcomes of increased height velocity without advanced bone age, advanced sexual maturation and often improvement in psychosocial parameters.

The most commonly used treatment regimen for boys with DP is supplementation with intramuscular depot preparations of a testosterone ester at a starting dose of 50 mg each month for 3–6 months; a further 3–6 months of treatment may be given, with dose increases as required (Table 7.12). High doses of testosterone should be avoided as this may induce acceleration of bone age and reduce adult height due to premature epiphyseal fusion. The total duration of treatment varies from 6 to 24 months with doses of 50–100 mg monthly; an increase in testicular size indicates gonadotropin release despite the negative feedback effects of the exogenous treatment. Boys with permanent HH will not have testicular growth.

Monitoring by serum testosterone increase (to mid-reference range 1-week post-injection), height velocity

Table 7.12 Medications used for the treatment of self-limited delay of puberty and permanent hypogonadism – males.

	Induction of puberty in boys		
Drug and formulation	**Isolated DP**	**Hypogonadism**	**Side effects and cautions**
Testosterone (T)[a]			Erythrocytosis, weight gain, prostatic hyperplasia. High doses can cause premature epiphyseal closure. Not for use in boys with bone age <10 years
T enanthate, cypionate and propionate. T enanthate has longer duration of effect than T propionate. IM injection	Not recommended before 13.5 years of age. Initial dose 50–100 mg every 4 weeks for 3–6 months. After review of response: repeated treatment with 25–50 mg increment in dose (not exceeding 100 mg)	Can initiate after age 12 years at 50 mg/month. Increase with 50 mg increments every 6–12 months. After reaching 100–150 mg monthly, decrease interval to every 2 weeks. Adult dose 200 mg every 2 weeks	All IM preparations: local side effects (pain, erythema, inflammatory reaction and sterile abscess). Priapism can occur in patients with sickle cell disease
T undecanoate IM injection	No data available	Adult dose is 1000 mg every 10–14 weeks	Very rarely, paroxysms of coughing and dyspnoea post-injection, ascribed to lipid embolism from the vehicle, hence not licenced in the USA
T gel. Transdermal preparations, applied topically at bedtime	No data available	Can be started when ~50% adult dose with IM T has been achieved. Adult dose: 50–80 mg daily	Local irritation. After applying, avoid close skin contact with others, especially females
	Treatment of fertility in boys and men[b]		
	Isolated DP	**Hypogonadism**	
Pulsatile GnRH s.c. pump	Not recommended routinely	Initial: 5–25 ng/kg/pulse every 90–120 minutes; increase to 25–600 ng/kg/pulse	Requires extensive experience. Most physiological form of replacement
hCG (SC or IM) plus recombinant FSH (SC)	Not recommended routinely	hCG dose: 500–3000 IU twice weekly, increased to every 2 days. Dose adjusted based on serum T levels. rhFSH dose: 75–225 IU 2–3 times weekly[c]	hCG: inflammation locally in the testis, may induce apoptosis of germ cells In hypogonadotropic hypogonadism with prepubertal onset, it is necessary to add FSH to induce testicular growth and spermatogenesis. No data on effects on future fertility

Source: Table modified and reprinted with permission from Palmert and Dunkel [56].

[a] Testosterone undecanoate PO tablets or anabolic steroids are not recommended for the induction of secondary sexual characteristics.

[b] Induction of fertility may be less successful in men who have lower baseline testicular volumes, have received previously testosterone treatment and have not previously received treatment with GnRH or gonadotropins.

[c] FSH pretreatment for 4 months may be beneficial in men with prepubertal testes [86].

and virilization is appropriate. The length of the polymorphic cytosine–adenine–guanine (CAG) trinucleotide repeats present in the AR gene is associated with AR activity, which may in part modulate response to testosterone therapy. A diagnosis of GH deficiency must be ruled out if height velocity does not increase on testosterone therapy. Testosterone esters are to be avoided when there is hepatic impairment or hypercalcaemia and should be used with caution in renal impairment. Preparations are generally well tolerated but side effects may include headaches, depression and androgenic effects such as acne. Oral testosterone undecanoate can cause wide variations in serum testosterone because of its short half-life and thus requires careful monitoring, although it has been successfully used for pubertal induction at a dose of 40–160 mg daily. Although anabolic steroids such as oxandrolone have been used historically for short-term improvement in height velocity, they are less effective in stimulating puberty and therefore no longer recommended for the management of DP.

DP is commonly seen in combination with idiopathic short stature (ISS) and such patients may present with concerns about short stature far outweighing those about DP. After exclusion of those patients with GH deficiency, for example, by the use of a primed GH provocation test, the treatment of GH-repleted DP patients with GH remains controversial: it has been approved by the US FDA for the treatment of ISS and height SDS of ≤ -2.25 for age but leads to only a modest increase in adult height and its use is not generally recommended.

Another potential pharmacological target in short boys with DP is inhibition of oestrogen biosynthesis from androgens using AIs. Epiphyseal closure is dependent on oestrogens and AIs could extend the time period of long bone growth. Some published data support this possible effect but, despite some reassuring recent data, safety concerns of the therapy do not recommend it.

Permanent Hypogonadism

Although sex steroid replacement is used in nearly all conditions of hypogonadism for initiation of male puberty, more complex and involved management including gonadotropin treatment may be required to achieve the development of secondary sexual characteristics and maximize the potential for fertility.

Hypogonadotropic Hypogonadism

In young men with a diagnosis of IHH, induction of puberty with sex steroid therapy is similar to that in self-limited DP but treatment can be started at a younger age (12 years) if the condition is confirmed. In some patients, it may not be possible initially to distinguish IHH from DP and treatment with testosterone may be delayed until 14 years. The starting dose of testosterone ester for IHH patients is commonly 50 mg but doses are gradually increased to full adult replacement levels over ~3 years (Table 7.12). Monitoring of response to treatment and possible side effects is required and therapy is likely to be required lifelong. After adult doses have been reached, maintenance therapy can be with IM testosterone, often as the longer-acting testosterone undecanoate (Nebido), topical or oral therapy.

Testosterone does not induce testicular growth or spermatogenesis in men with HH, as this is dependent on high intra-testicular concentrations of testosterone produced by LH-stimulated Leydig cells in conjunction with FSH acting on Sertoli cells. Induction of fertility requires treatment with either pulsatile GnRH or exogenous gonadotropins. Data from the last 5–10 years on a variety of regimens have been published, with treatments varying by indication, underlying diagnosis and severity of hypogonadism. Fertility outcomes vary, with poorer responses in patients with signs of absent mini-puberty (prepubertal testes, cryptorchidism and/or low inhibin B). Genetic diagnoses may guide therapy: treatment of patients with *ANOS1* mutations can be more difficult as they may have defects at several levels of the HPG axis; patients with IHH due to *GnRHR* mutations may be better treated with hCG and FSH than pulsatile GnRH.

A subset of adolescent patients with IHH will have had a spontaneous onset of pubertal development that has arrested. In such patients, monotherapy with hCG can be tried for both completion of pubertal development and induction of fertility. FSH can be added if there is persistent azoospermia after 6–12 months of treatment. In adolescent males without puberty, induction of puberty either with hCG monotherapy or with combinational therapy of hCG + rFSH leads to better testicular growth and fertility outcomes than treatment with testosterone alone. A combined regime of hCG + FSH has greater potential efficacy in the induction of spermatogenesis than monotherapy with hCG.

Timing of treatment is important, as FSH pretreatment may theoretically optimize the Sertoli cell population before exposure to hCG or GnRH and thus has the potential to improve fertility outcomes. Although the optimal regimen in severe cases, i.e. those with testicular volume of <4 mL, is unknown, FSH pretreatment followed by GnRH or combination hCG and FSH treatment may maximize the potential for fertility [86]. Earlier age of treatment to induce spermatogenesis may also be beneficial in increasing the capacity for and speed of sperm production once fertility is desired, but assisted reproductive technologies may still be required.

A small number of individuals with IHH are diagnosed in infancy. Postnatal HPG axis activation in boys, which results in testicular activation and proliferation of

Sertoli cells during this period, has a role in the development of reproductive capacity. The association of testosterone concentrations at three months of age with early penile growth as well as involution of the penis and scrotum in boys with IHH in infancy suggests a role for postnatal testosterone in 'stabilizing' male genitalia. Analogous to true puberty, androgens secreted early in life may also have effects on linear growth, skeletal development, body composition and psychosexual development.

Hormone therapy has thus been advocated for penile growth and testicular descent in infant boys with IHH or KS. Decreased serum androgen bioactivity has also been reported in infant boys with at least one undescended testis. Neonatal treatment with testosterone can be used to correct micropenis in both central and primary hypogonadism. Standard therapy is with either IM testosterone enanthate 25 mg every 3–4 weeks for 3 months or topical therapy with either 5% testosterone cream or DHT. Management of cryptorchidism is with surgical correction; the use of hCG or GnRH therapy adjuncts does not provide additional benefit.

These therapies will not address the microorchidism seen in a male infant with IHH. A small number of studies of infants with IHH have used recombinant LH and FSH treatments to increase both penile length and testicular volume. Outcomes included improved testicular size and function (measured with inhibin B and AMH) but it is not known if such therapy will improve the response to pubertal treatments or fertility outcomes in men with IHH. Concerns remain about the possible deleterious effects of hCG on germ cells in cryptorchid testes in infants as its use has been associated with smaller testicular volumes and higher FSH concentrations in adulthood.

At the other end of the spectrum, patients can exhibit reversal of their phenotype during treatment. This phenomenon is being increasingly recognized in up to 20% of IHH cases [87]. Reversal phenotypes have been demonstrated with several CHH genotypes, including *ANOS1*, *FGFR1*, *GnRHR* and *PROKR2*. In these patients, very late activation of pulsatile gonadotropin secretion (due to late activation of the GnRH pulse generator or gonadotroph responsiveness) occurs such that gonadotropin secretion improves with time. Awareness of this is important as a 'trial off treatment' can be used intermittently to assess requirements for maintenance therapy. These cases may relapse off treatment and need ongoing monitoring. Patients with acquired HH, usually secondary to tumours or other structural lesions of the hypothalamic–pituitary axis or haemochromatosis, require treatment of their underlying condition with sex steroid or gonadotropin therapy depending on their specific requirements.

Hypergonadotropic Hypogonadism

The majority of males affected with Klinefelter syndrome will enter puberty spontaneously at a normal age, although DP may be seen in those with a more complex karyotype (48,XXYY; 48,XXXY; 49,XXXXY). Sex steroid replacement is therefore not normally required for these patients at the start of puberty but testosterone concentrations become increasingly deficient by Tanner stages 4–5, possibly as a result of secondary regression. However, only 10% of boys aged 10–14 years with Klinefelter syndrome have been diagnosed and many patients only come to the attention of an endocrinologist in later adulthood. These patients present difficult management decisions in terms of optimizing fertility outcomes, mainly relating to timing of interventions. For patients with Klinefelter syndrome requiring treatment due to falling testosterone concentrations, haematocrit, bone density, patient well-being or sexual function, low-dose sex steroid replacement is the most commonly used therapy (Table 7.12). Testicular sperm extraction and cryopreservation can be considered, even in adolescence before testosterone treatment and before the progressive seminiferous tubule degeneration that occurs in Klinefelter syndrome has had an irreversible impact on sperm production. Unfortunately, the most invasive (and successful) sperm retrieval techniques have the potential to cause the most testicular damage and so would ideally be reserved for those men actively desiring fertility. Balancing these opposing factors and giving clear information to young men who may not yet be concerned about their future fertility options in order that they might make informed choices are challenging.

The treatment for induction and maintenance of puberty of anorchic young men secondary to congenital absence, vanishing testis syndrome or failed orchidopexy is similar to that in boys with IHH. Androgen replacement should be commenced at a low dose with incremental doses. IM testosterone esters administered monthly are the treatment of choice for pubertal induction, with testosterone gel via calibrated dispenser or 3 monthly intramuscular depot injections of testosterone undecanoate 1 g used for long-term maintenance therapy (Table 7.12).

Females

Self-Limited Delayed Puberty

The options for management of female patients with DP include monitoring with reassurance or therapy using low-dose sex steroids to initiate pubertal development. Initial short-term therapy should be regularly reassessed and discontinued once puberty is progressing (Table 7.13).

Table 7.13 Medications used for the treatment of self-limited delay of puberty and permanent hypogonadism – females.

	Induction of puberty in girls		
Drug and formulation	**Isolated DP**	**Hypogonadism**	**Side effects and cautions**
Oestrogens	Not recommended before 13 years of age		
Transdermal 17b-oestradiol, e.g. Evorel 25	Overnight patch: initial dose, 3.1–6.2 µg/24 h (1/8–1/4 of 25 µg 24-h patch); increase by 3.1–6.2 µg/24 h after 6 months[a]	Starting dose as for DP; increase by 3.1–6.2 µg/24 h until 1 full Evorel 25 patch continuously[b] Then maintenance with adult COCP or HRT	Patches may be difficult to use and fall off, especially if cutting whole patches into smaller fractions Reactions to adhesive Inter-individual variation in dose response
Oral 17b-oestradiol (oestradiol valerate)	0.5 mg (1/2 tablet) alternate days or 5 µg/kg of body weight daily; increase to 0.5 mg (1/2 tablet) or 10 µg/kg daily after 6–12 months	Starting dose as for DP, increase by 5 µg/kg of body weight every 6–12 months until dose of 1 mg (1 tablet) daily Then maintenance with adult COCP or HRT	Inter-individual variation in dose response
Oral ethinylestradiol (2 µg tablets)	2 µg daily, increase to 4 µg after 6 months if required	2 µg daily, increase by 2 µg every 6 months until 10 µg Then maintenance with adult COCP or HRT	High cost Liver toxicity, increased levels of some plasma-binding proteins. Potential increased risk of hypertension and VTE Worse growth profile
Progestins	Not applicable	Introduced once breakthrough bleeding or 2+ years of continuous oestrogen	
Norethisterone		5 mg bd	More androgenic, increased risk of dysmenorrhoea
Utrogestan		200 mg once daily	
Medroxyprogesterone acetate		5 mg once daily	
Combination preparations		e.g. Evorel Sequi, Elleste Duet	
	Treatment of fertility in women		
Pulsatile GnRH s.c. pump	Not applicable	Requires extensive experience, treatment only within specialist centres. Most physiological form of replacement	
hCG (SC or IM) plus recombinant FSH (SC)	Not applicable	Requires extensive experience, treatment only within specialist fertility centres	

[a] Adjustments for body weight may be required, published advice on cutting patches available [88].
[b] Once changed from overnight to all day use, patches to be changed twice weekly. COCP, combined oral contraceptive pill; HRT, hormone replacement therapy; VTE, venous thromboembolism.

Permanent Hypogonadism

More intensive and long-lasting therapy is required in cases of permanent hypogonadism. Goals of treatment are induction of secondary sexual characteristics, development of reproductive capacity and increasing adult height. Once puberty is complete, ovulation and pregnancy can be achieved by pulsatile GnRH administration or combination gonadotropin therapy.

When oestrogen therapy is required to induce pubertal development, the dose and timing should be aimed at simulating normal growth and development of secondary sex characteristics taking account of the individual's desire to begin puberty and also of the family history of age at the onset of puberty. Doses should be adjusted according to the needs and priorities of the individual. Response to therapy should be monitored by the development of secondary sex characteristics, bone maturation, height velocity and uterine volume, with additional monitoring of blood pressure and bone density.

Both in young women with Turner syndrome and combined pituitary hormone deficiency, oestrogen therapy should be coordinated with the use of GH.

Previous practice in Turner syndrome tended towards delaying oestrogen therapy until the mid-teens in order to optimize growth promotion with GH but more recent studies point to potential benefits from treatment with combined very low-dose oestrogen and GH from an early age for final height and potentially for other areas including cognitive development and uterine maturation. It remains unclear as to the best time to start oestrogen therapy in young women with Turner syndrome but the current consensus is that induction of puberty should not be delayed in order to promote linear growth [72].

Additionally, while ethinylestradiol has traditionally been the oestrogen of choice for pediatric patients, 17β-oestradiol in transdermal, gel or oral form displays a better risk profile in terms of growth restriction, liver toxicity and vascular side effects. Data from women with combined pituitary hormone deficiencies receiving combined oestrogen and GH treatment indicate a markedly greater impairment of GH-mediated IGF1 synthesis with ethinylestradiol than with 17β-oestradiol. Uterine development may also be impaired with the use of ethinylestradiol as compared with 17β-oestradiol. Conjugated equine oestrogens have been used, but formulations vary in biological potency and are best avoided in view of reports of increased cardiovascular risks in postmenopausal women.

Oestrogen therapy should be initiated at a low dose (one-eighth to one-quarter of the adult dose) and increased gradually (at intervals of 6–12 months) [89]. Doses can then be adjusted to the response (Tanner stage, bone age and uterine growth) or by ultrasensitive oestradiol assay with the aim of completing feminization gradually over a period of 2–3 years (Table 7.13).

A progestin such as oral medroxyprogesterone acetate should be added either if more than one episode of significant breakthrough bleeding occurs or after 24–36 months of oestrogen therapy in order to establish menstrual cycles with a frequency of at least every 2–3 months to prevent endometrial hypertrophy.

Individuals with Turner syndrome who have functioning ovaries and who progress through puberty spontaneously should receive contraceptive and genetic counselling. Ovulatory function should be documented (FSH and LH measurements) because a perimenopausal pattern of anovulation can lead to endometrial hyperplasia.

For women with gonadotropin deficiency, pulsatile GnRH treatments provide information on the potential to induce ovulation and can be considered when fertility is desired. For patients with primary gonadal failure, fertility may be possible in some cases by assisted reproductive procedures. In girls at high risk for the development of primary ovarian insufficiency, the option of oocyte or ovarian tissue cryopreservation should be considered.

In both genders, optimization of vitamin D and calcium intakes are advisable to help bone mineral density acquisition.

Conclusions

Puberty is the period of sexual maturation when the transition to adult reproductive capacity, body composition and adult height occurs. Its biological control is complex and involves multiple endocrine systems interacting in an ordered and progressive pattern. The origins of these biological processes begin early in fetal life, and fetal and neonatal developments are important to allow puberty to occur normally in adolescence. The mystery of what induces the dormancy of the HPG axis after the mini-puberty and what triggers the release of this 'puberty brake' remains unanswered. There are multiple influences on the timing of puberty in the general population and appropriate age cut-offs for early and late puberty in different ethnic groups may vary.

A wide variety of genetic, epigenetic and environmental factors affecting different aspects of the HPG axis at different time periods in fetal and postnatal life may result in precocious, delayed and disordered puberty. Benign variants of puberty are common and are closely associated with variants in childhood growth and development. Precocious and DP are both frequent problems presenting to the pediatric endocrinologist. While PP is often idiopathic and the most common underlying condition in delayed onset of puberty is self-limited (or constitutional) DP, other pathological causes may underlie these conditions and must be excluded.

In PP, the differential diagnoses of tumours, CAH and rarer peripheral causes need to be considered. In DP, HH, either functional or permanent, and primary hypogonadism must be excluded. In particular, distinguishing between self-limited DP and permanent HH in adolescence remains difficult but the latter can be diagnosed in infancy if the suspicion arises.

Management of adolescents with pubertal disorders is dependent on the underlying cause. Expectant observation is appropriate in benign variants of puberty and in those with milder forms of precocious or DP who are not predicted to have negative outcomes from their condition. Treatment of more significant precocious or DP involves medication to block or induce activity of the HPG axis, while more complex and involved management is required in patients with complex peripheral PP or permanent hypogonadism. Achievement of fertility in patients with central hypogonadism requires therapy with gonadotropins. The management of infants diagnosed with permanent central hypogonadism is an area for future research. Genetic testing may inform diagnosis of associated syndromic features, natural history of the condition and inheritance in family members.

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8

The Thyroid Gland

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KEY LEARNING POINTS

- Fetal secretion of thyroid hormones commences at the end of the first trimester, and T3 and T4 concentrations rise slowly up to term.
- The fetus is particularly dependent on maternal thyroxine supply in the first trimester.
- Insufficient thyroid hormone supply *in utero* leads to physical and neurodevelopmental delay in the infant, which can be profound.
- Screening for congenital hypothyroidism (CH) has been a significant public health success with near elimination of cretinism where introduced.
- Infants diagnosed with CH should be treated immediately with the aim of rapidly normalizing thyroid function.
- Thyroid dysgenesis is usually a sporadic disease, but dyshormonogenesis is usually attributable to mutations in one of the key genes controlling thyroid hormone biosynthesis.
- Autoimmune thyroid disease is the most common cause of acquired thyroid disease.
- Autoimmune hypothyroidism (usually Hashimoto's) can present insidiously, and replacement with thyroxine should be gradual.
- Autoimmune hyperthyroidism (usually Graves') is usually treated with methimazole (or carbimazole) as first line and has an overall remission rate of 30%.
- Propylthiouracil is not recommended as first-line treatment due to hepatic side effects.
- Radioactive iodine is not recommended for children <5 years and should be used with caution in those aged 5–10 years.
- Thyroidectomy should be performed only by a pediatric surgeon with a high throughput of cases and experience.
- Iodine insufficiency is common in Europe and has implications for thyroid status and childhood cognitive development.
- Assay interference and defects in thyroid hormone action, metabolism and transport can lead to elevated thyroid hormone concentrations with unsuppressed TSH.

Section 1: Development of the Thyroid Axis

Thyroid Gland Development

Thyroid Morphogenesis

Thyroid morphogenesis begins with the specification of a thyroid domain along the anterior–posterior axis of the ventral foregut endoderm. By 22 days postfertilization, a midline placode close to the tongue base defines the median anlage that will contribute the majority of the thyroid follicular cell mass. This evaginates from the endodermal epithelium by day 24 postfertilization (budding), and the thyroid precursor cells migrate caudally in close proximity to the aortic sac between 28 and 48 days postfertilization, proliferating and expanding laterally during this process (lobulation).

The ultimobranchial bodies originating from the fourth pharyngeal pouch deliver the parafollicular or C cells, fusing with the median anlage-derived cells around day 44. Although traditionally thought to originate from the neuroectoderm, C cells may also have an anterior endodermal origin. By the time the thyroid reaches its final position anterior to the trachea, at embryonic day 48, the final shape of the gland is established, and terminal differentiation begins (Figure 8.1). This requires polarization and adhesion of individual thyroid follicular cells to form follicles and functional changes whereby polarized thyroid follicular cells acquire the capacity to synthesize thyroid hormone within the follicular lumen [1–4].

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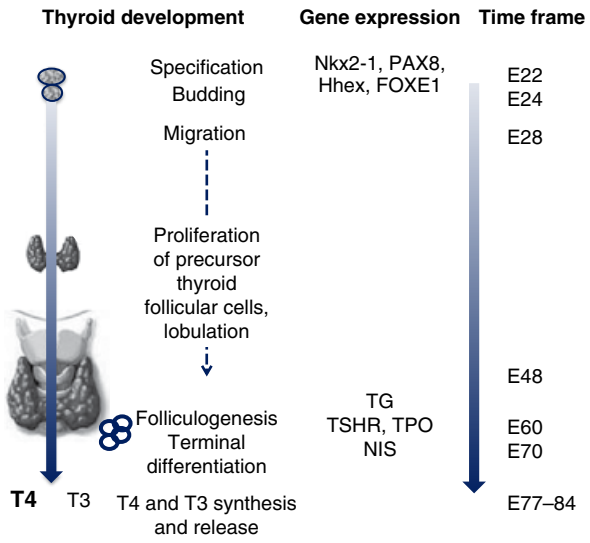

Figure 8.1 A summary of the key steps in thyroid gland morphogenesis and their approximate embryological timing, together with key gene expression coinciding with or preceding these processes.

Folliculogenesis beginning around day 60 postfertilization is accompanied by expression of *Tpo* and *Tshr*. By 70 days postfertilization, a completely formed thyroid gland expresses *Slc5a5*, thus acquiring the capacity to accumulate iodide and synthesize thyroid hormones. Fetal thyroid hormones can be detected in the thyroid from gestational week 11 and in fetal blood from gestational week 12 [2, 4–7].

Molecular Control of Thyroid Development

Known Transcription Factor Involvement

Human thyroid development is recapitulated in murine models that have significantly contributed to understanding thyroid morphogenesis. Developing murine thyroid is defined by the expression of four key transcription factors, paired box 8 (*Pax8*), NK2 homeobox 1 (*Nkx2-1*, previously known as TTF1), haemopoetically expressed homeobox (*Hhex*) and forkhead box E1 (*Foxe1*, previously known as TTF2). Although each of these transcription factors also has distinct roles in extrathyroidal tissues, their combined expression to mediate organogenesis is observed only in epithelial thyroid follicular cells [5].

Murine *Nkx2-1, Pax8* and *Hhex* are initially expressed as independent proteins, but by E10 they participate in a network of reciprocal regulatory interaction. Although all four transcription factors are crucial for embryonic thyroid development, *Foxe1* is located downstream in the thyroid transcription factor hierarchy, requiring Pax8 for the onset of expression, and, in human thyroid primordium, FOXE1 protein is detected later than PAX8 and NKX2-1 [4, 5]. Expression of NKX2-1 and PAX8 alone in response to FGF2 and BMP4 signalling *in vitro* was sufficient to drive differentiation of murine and human pluripotent stem cell-derived endodermal precursors into thyroid follicular structures capable of producing thyroid hormones when exposed to thyrotropin [8, 9].

As well as mediating the formation of the thyroid bud and survival of thyroid progenitor cells, these transcription factors drive functional differentiation, regulate the expression of genes involved in thyroid hormone biosynthesis and play a role in the maintenance of the mature thyroid gland, although the role of HHEX in the latter context is less clearly understood. FOXE1 also has a particular involvement in thyroid migration. Mutations in *NKX2-1*, *PAX8* and *FOXE1* are all associated with thyroid dysgenesis (TD) in humans [5, 7, 10–13].

Novel Candidate Genes

The process of thyroid embryogenesis remains incompletely understood, and murine and zebrafish models have contributed to hypotheses for putative contributing mechanisms. Extrinsic factors may be implicated, in common with development of other endodermal-derived organs (e.g. the liver and pancreas) that require permissive signals from the adjacent mesoderm. The close proximity of the anterior foregut endoderm and precursor thyroid follicular cells to the precardiac mesoderm and aortic sac would enable similar interactions and this is supported by murine and zebrafish studies.

Components of the fibroblast growth factor (FGF) signalling pathway (Hand2 and FGF1, 2 and 8) [14] and Tbx1, the candidate gene for DiGeorge syndrome and a transcriptional regulator of FGFs, are examples of mesoderm-derived, non-cell autonomous factors likely to play a role in thyroid development [15, 16]. A tightly controlled anti-apoptotic network is also crucial for thyroid morphogenesis, as evidenced by the loss of the developing thyroid bud in Nkx2-1, Pax8 and Hhex null murine embryos [17]. In keeping with the importance of this pathway, the bcl2-like gene (bcl2l) appears to control a caspase-3-dependent apoptotic mechanism, which is crucial for thyroid cell survival in zebrafish [18].

Human loss-of-function variants in genes known to contribute to thyroid morphogenesis in animal models have been described in association with TD, but penetrance is highly variable. Such genes include the Notch ligand JAG1, the thyroid and cardiac transcription factor Nkx2-5 and NTN1, which is implicated in zebrafish aortic arch artery formation and thyroid morphogenesis, supporting a role for the embryonic vasculature in providing guiding tracks for bilateral thyroid growth [19–22].

Thyroid Hormone Biosynthesis

Thyroid follicles comprise a monolayer of polarized thyrocytes with the basolateral surface facing the bloodstream and

the apical surface apposed to a central, spherical, lumen in which thyroid hormones are synthesized. Production is stimulated by TSH and requires an adequate supply of iodine and intact biosynthetic machinery comprising transporter molecules, enzymes and thyroglobulin (Figure 8.2).

The thyroid gland maintains a concentration of free iodide 20–40 times higher than that of plasma under physiological conditions and iodide uptake occurs across the basolateral membrane of the thyroid follicular cell via the sodium–iodide symporter (NIS) encoded by *SLC5A5*. NIS is a 13 transmembrane domain glycoprotein that actively co-transports one iodide ion against its electrochemical gradient together with two sodium ions along a sodium gradient generated by the ouabain-sensitive Na+/K+-ATPase [23, 24]. Iodide efflux at the apical membrane is probably mediated by more than one iodide transporter [25] but their molecular identities remain a subject of debate.

Pendrin, encoded by *SLC26A4*, is a multifunctional anion transporter expressed at the thyrocyte apical membrane capable of exchanging chloride for either iodide or bicarbonate. Its location and the association of biallelic pendrin mutations in some patients with goitre and impaired iodide organification support a role for pendrin in apical iodide transport [26–28]. However, individuals with biallelic mutations in pendrin may exhibit normal thyroid function under conditions of normal iodide intake [29], and the pendrin null mouse remains euthyroid even when deprived of dietary iodine, suggesting the involvement of alternative transporters [30].

Recently, anoctamin 1 (TMEM16A), a calcium-activated anion channel, has been detected at the apical membrane of thyrocytes and functional studies suggest that it may play a more dominant role in mediating iodide efflux [31].

Once in the follicular lumen, iodide is oxidized and then organified into selected tyrosyl residues of thyroglobulin, a very large (660 kDa) secreted glycoprotein that serves as the matrix for thyroid hormone synthesis. Four main hormonogenic acceptor tyrosines have been identified at positions 5 (exon 2), 1291 (exon 18), 2554 (exon 44) and 2747 (exon 48) [32–34]. The incorporation of iodine results in the formation of mono- and diiodotyrosines (MIT, DIT) that undergo pairwise phenoxy-ether bond formation to generate iodothyronines (predominantly T4, with smaller amounts of T3 and reverse T3).

Oxidation and organification of iodide and the subsequent coupling reaction are hydrogen peroxide-dependent and catalysed by the thyroid-specific enzyme thyroid peroxidase (TPO), a haem peroxidase anchored via a C-terminal transmembrane domain at the apical membrane surface of thyroid follicular cells [34, 35].

Triiodothyronine (T3) and thyroxine (T4) are stored in the colloid until thyroglobulin is taken up by thyroid follicular cells through macro- and micropinocytosis and digested in lysosomes. Secretion of thyroid hormones into the bloodstream at the basolateral membrane (predominantly T4, since most T3 is produced by enzymatic deiodination of T4 in peripheral tissues) is mediated at least in part by the monocarboxylate transporter 8 (MCT8) [36]. Uncoupled MIT and DIT are subject to NADPH-dependent reductive deiodination by

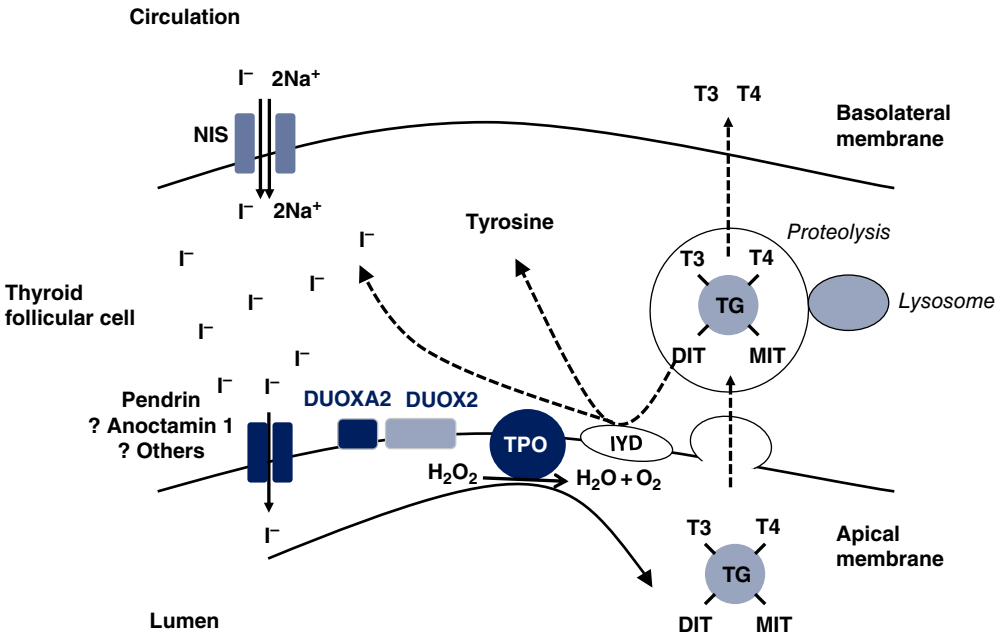

Figure 8.2 A summary of the key steps in thyroid hormone biosynthesis in the thyroid follicular cell. Mutations in the genes encoding all of the protein molecules shown (with the exception of anoctamin or other putative apical iodide transport proteins) have been reported in association with dyshormonogenesis and congenital hypothyroidism.

iodotyrosine dehalogenase (IYD, previously known as DEHAL1) leading to formation of free iodide and tyrosine, both of which can be reutilized in hormone synthesis [34, 37, 38].

Hydrogen peroxide (H_2O_2) is the essential electron acceptor for the iodination and coupling reactions. It is produced mainly at the apical membrane of follicular thyroid cells by NADPH oxidase dual oxidase 2 (DUOX2, previously known as THOX2) and its accessory protein dual oxidase maturation factor 2 (DUOXA2). There is a high level of functional redundancy in the H_2O_2-generating system, and other enzymes capable of H_2O_2 production in the thyrocyte include the dual oxidase 1 (DUOX1, previously known as THOX1)/dual oxidase maturation factor 1 (DUOXA1) system. DUOX2 is thought to be the main H_2O_2-generating enzyme in the thyroid on the basis of its higher thyroidal expression levels and the recognition that human mutations in both *DUOX2* and *DUOXA2* but not *DUOX1* have been implicated in congenital hypothyroidism (CH). Additionally, in murine models, only *DUOX2* loss of function is associated with hypothyroidism; *DUOX1* null mice are euthyroid, and the role of DUOX1 in thyroid biology remains unclear [39]. Heterodimerization of a DUOXA (DUOXA1 or DUOXA2) subunit with a DUOX subunit is essential for the maturation, membrane translocation and function of the respective DUOX isoenzymes [40].

Iodine is the main environmental factor influencing thyroid hormone biosynthesis and iodine deficiency is the commonest cause of CH worldwide. Excess iodide can inhibit thyroid hormone synthesis acutely in a normally functioning thyroid gland (the Wolff–Chaikoff effect) [41] but the biochemical mechanism underlying this remains controversial. It may be partly due to the fact that excess iodide results in the formation of organic iodocompounds such as 2-iodohexadecanal that inhibit TPO [42]. Escape from the Wolff–Chaikoff effect occurs after a few days as a consequence of decreased thyroidal inorganic iodine concentration secondary to downregulation of the NIS [43].

The Hypothalamic–Pituitary–Thyroid Axis

Positive Regulation of Thyroid Hormone Synthesis

Thyroid hormone biosynthesis is positively regulated by the actions of hypothalamic thyrotropin-releasing hormone (TRH) and pituitary thyroid-stimulating hormone (TSH). TRH is synthesized as a prohormone in the paraventricular nucleus (PVN) of the hypothalamus. It matures into the TRH tripeptide amide (pGlu-His-ProNH2) following post-translational cleavage by prohormone convertases PC1/3 and 2. Following axonal transport to the median eminence, TRH reaches the thyrotrophs of the anterior pituitary gland via the hypothalamic portal vein where it binds the TRH receptor (TRHR). Residual extracellular TRH is rapidly degraded by the enzyme pyroglutamyl peptidase II (PPII) [44, 45].

TRHR is a G-protein-coupled receptor, which activates a Gq/11-dependent pathway involving mobilization of intracellular calcium and activation of protein kinase C. Normal TRHR expression may require the membrane glycoprotein IGSF1 although the mechanism underlying this remains unclear [46]. Activation of the TRHR upregulates transcription of the TSH alpha- (αGSU) and beta-subunit genes (*CGA* and *TSHB*) as well as mediating conjugation of TSH alpha and beta subunits and regulating secretion of heterodimeric TSH and its glycosylation to confer normal bioactivity.

TSH bioactivity is influenced by the post-translational incorporation of oligosaccharide, especially at Asn-76 and Asn-102 of the alpha subunit and Asn-43 on the beta subunit such that TSH with a high sialic acid content exhibits decreased bioactivity and increased half-life [47, 48]. TSH binds a G-protein-coupled receptor, TSH receptor (TSHR) in the thyroid, for which cyclic AMP (cAMP) is the major second messenger following activation, which stimulates both follicular cell growth and thyroid hormone synthesis and release. The TSHR interacts with both Gαs and Gαq in the thyroid and is capable of activating both adenylyl cyclase and phospholipase C downstream signalling cascades [49].

Negative Regulation of Thyroid Hormone Synthesis

As well as stimulating positive regulation of thyroid hormone biosynthesis, a sensitive negative feedback mechanism operates within the hypothalamic–pituitary–thyroid (HPT) axis. This enables circulating concentrations of thyroid hormones to be maintained within an individually unique set point within a population reference range. In the hypothalamus, thyroid hormone is taken up into the brain from the cerebrospinal fluid (CSF) in the third ventricle or from blood vessels in the median eminence by type 2 deiodinase (DIO2)-expressing tanycytes or astrocytes, respectively. DIO2 converts T4 to T3 that then enters the TRH neurons and binds thyroid hormone receptors [50–52].

A shorter T3-mediated negative feedback loop operates in the anterior pituitary; TSHR expression has also been demonstrated in pituitary folliculostellate cells, leading to speculation that paracrine signalling in the pituitary may also contribute to negative feedback [50, 53]. Intracellular delivery of thyroid hormones requires active transport probably mediated by MCT8 for uptake into neuronal cells and across the blood–brain barrier. Organic anion-transporting polypeptide 1c1 (OATP1C1) may play a role in transport into astrocytes [50–52].

The transport mechanism for pituitary thyroid hormone uptake remains poorly defined.

Thyroid hormones downregulate hypothalamic transcription of the pro-TRH and PC1/3 and PC2 genes in the hypothalamus [54, 55], thereby decreasing levels of mature TRH and inhibiting transcription of *CGA* and *TSHB* in the anterior pituitary [56–58]. Additional modulators of TSH secretion include hypothalamic dopamine and somatostatin (inhibitory), feeding behaviour, glucocorticoids, severe illness, cold and circadian rhythm [59].

Thyroid Hormone Transport

Extracellular Transport

Thyroid hormones are lipophilic and poorly water-soluble so more than 99% of circulating T4 and T3 bind reversibly to three principal plasma binding proteins, thyroxine-binding globulin (TBG), transthyretin (TTR) and albumin with tight, non-covalent bonds. Only the free hormone (0.03% of total T4 and 3% of T3) is available for cellular uptake, but free and bound hormones exist in rapid equilibrium [60]. The large binding capacity of these plasma proteins for thyroid hormones enables free hormone levels to be buffered against depletion by active uptake and metabolism, thus maintaining a stable free hormone concentration for tissue uptake.

Association with binding proteins also shields the hydrophobic thyroid hormones from their aqueous environment and enables an even uptake of thyroid hormone throughout different tissues [61]. T4 is bound more strongly than T3 and therefore the relative half-lives of the two hormones are different (6 days for T4, 2.5 days for T3), and T3 concentrations respond more rapidly to altered hormone delivery to the bloodstream [60].

Thyroid Hormone Transport Proteins

TBG is a 54kDa glycoprotein synthesized by the liver and encoded by the *TBG* gene on the X chromosome (Xq21–Xq22). TBG is the principal thyroid hormone carrier protein, binding ~75% of circulating T4 and T3. Despite lower circulating concentrations (0.27×10^{-6} M) compared with TTR (4.6×10^{-6} M) and albumin (640×10^{-6} M), the high thyroid hormone binding affinity of TBG results in it transporting the majority of bound T3 and T4 [60].

TTR is a 55 kDa tetrameric protein synthesized predominantly by the liver and choroid plexus; in humans it transports only 20% of plasma T4, although it is the main thyroid hormone binding protein in CSF [62].

Human serum albumin, the most abundant plasma protein, is synthesized in the liver and exported as a single non-glycosylated 67 kDa chain. Its extraordinary ligand-binding capacity enables it to provide a depot for a wide variety of compounds, including haem, drugs and non-esterified fatty acids as well as 15% of circulating thyroid hormones [63]. Albumin has the lowest T4 affinity and fastest T4 release time of the major T4-binding proteins. Therefore it may provide an important fast-response reservoir for the hormone during capillary transit, promoting quick exchange of T4 within tissue sites.

Intracellular delivery of free hormones requires active transport by specific membrane transporters, including MCT8, MCT10 and OATP1C1. MCT8 is highly specific for T4 and T3 transport. MCT10 is a transporter of aromatic amino acids and transports T3 with greater and T4 with lesser efficiency than MCT8. The OATPs are proteins with 12 transmembrane domains that mediate transport of amphipathic organic compounds, and, of all 3 transporters, human OATP1C1 has the highest affinity for T4.

MCT8 exhibits a broad tissue distribution in the brain, liver, kidney, heart, thyroid and placenta. It is critical for thyroid hormone transport in the central nervous system (CNS) and is the only thyroid transporter with known pathogenic mutations in humans. After transport across the blood–brain barrier, T4 is taken up into astrocytes, where type 2 deiodinase (DIO2) converts it to T3. MCT8 facilitates both transport of T4 and T3 across the blood–brain barrier and T3 uptake by neurons. This is crucial for thyroid hormone to exert its effects since neuronal cells lack DIO2 and cannot convert T4 to T3 themselves. MCT8 also plays a role in thyroid hormone secretion from the thyroid gland. In rodents, OATP1C1 is highly enriched in astrocytes as well as the endothelial cells of the brain capillaries where it may mediate transport of T4 into astrocytes for subsequent deiodination to T3. In humans, OATP1C1 expression in capillary endothelial cells is weak, and its predominant role is likely to be in facilitating T4 uptake in astrocytes; MCT10 is expressed in the skeletal muscle, kidney, intestine and hypothalamus but its role in T4 transport also remains unclear [51, 52, 64, 65].

Thyroid Hormone Metabolism

The iodothyronine deiodinases belong to the selenoprotein family of enzymes that regulate local tissue availability of thyroid hormones by generating or catabolizing thyroid hormones. Type 2 deiodinase (DIO2) generates T3 by outer ring deiodination of T4. Type 3 deiodinase (DIO3) catalyses inner ring deiodination, thus inactivating T3 by converting it to 3,3′-diiodothyronine (T2) or preventing T3 synthesis from T4 by converting T4 to 3,3′5′-triiodothyronine (reverse T3). Type 1 deiodinase (DIO1) carries out inner or outer ring deiodination to

generate T3, rT3 or T2, depending on the substrate (Figure 8.3a). DIO1 is expressed in the liver, kidney and thyroid gland. DIO2 is expressed in brown adipose tissue and skeletal muscle; DIO3 is expressed in skin, uterus, placenta and fetal tissues; and both enzymes are expressed in the CNS (DIO2 in astrocytes, DIO3 in adjacent neurons), cochlea, retina and skeleton.

DIO1 contributes to plasma T3 production and has a major role in the clearance of reverse T3. DIO2 is more efficient than DIO1 and is crucial for modulating intracellular TH availability in response to physiological need. Under conditions of iodine deficiency or hypothyroidism, DIO2 activity is markedly upregulated, which is particularly important in protecting tissues from the adverse effects of hypothyroidism by mediating local conversion of T4 to T3. DIO3 degrades T3 and synergizes with DIO2 during fetal development, with high DIO3 expression, protecting the fetus from excess T3 exposure; at birth, DIO2 expression increases while DIO3 expression declines, triggering postnatal tissue maturation. In critical illness, alterations in deiodinase activity, including re-expression of DIO3, result in complex changes of thyroid hormone metabolism [66, 67].

Thyroid Hormone Action

Thyroid Hormone Receptor Isoforms

The genomic effects of thyroid hormone are mediated by the nuclear thyroid hormone receptors (TRs) α and β, encoded by the *THRA* and *B* genes on chromosomes 17 and 3, respectively. Alternative splicing and use of a different tissue-specific promoter (TRβ) generates multiple protein isoforms (including TRα1, α2, β1 and β2 in humans), which exhibit differing tissue distribution (Figure 8.3b). TRα1 and the two N-terminal variants of TRB bind T3 and act as T3-inducible transcription factors in humans. These isoforms share high sequence homology in the DNA and T3-binding domains but differ in length and amino-terminal domains. The C-terminal TRα variant (TRα2) fails to bind T3 and its physiological role remains unclear [68].

The effects of thyroid hormone are mediated by receptor interaction with specific DNA response elements (TREs), usually located in the promoter regions of target genes, to modulate their transcription in a ligand-dependent manner [69]. TRs bind preferentially to TREs as a heterodimer with the retinoid X receptor (RXR).

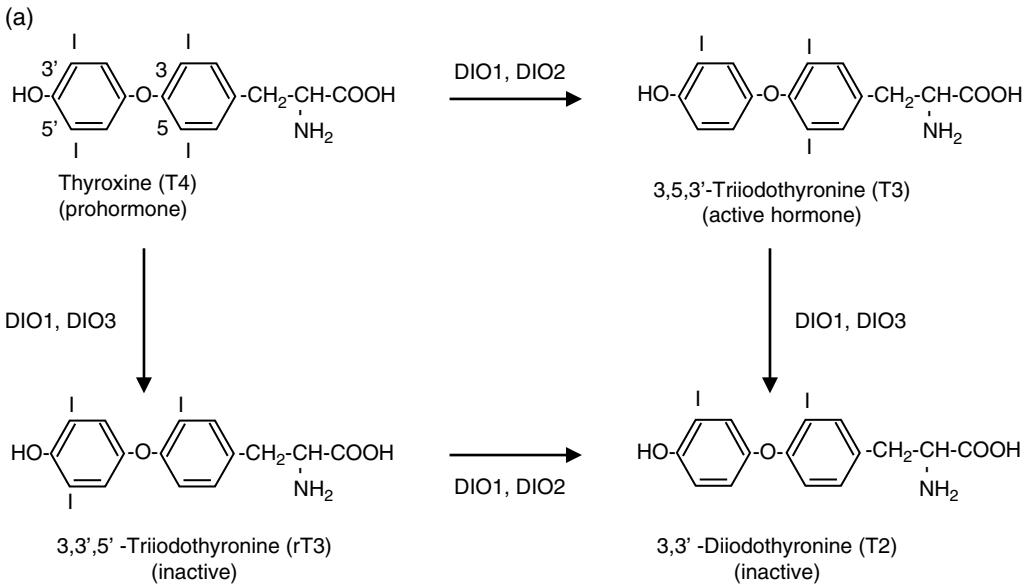

Figure 8.3 A panel of four illustrations: (a) The deiodination of thyroid hormones. (b) The human thyroid hormone receptor alpha and beta splice variants α1, α2, β1 and β2 with homologous regions shaded in the same colour and functional domains labelled. All except TRa2 bind T3 hormone. (c) A putative mechanism for the transcriptional regulation of positively regulated genes by thyroid hormone receptors; TR-RXR heterodimers bind specific sequences (TREs) in the regulatory region of target genes. In the absence of hormone, the unliganded receptor recruits corepressors (e.g. NCoR or SMRT) and histone deacetylases, which maintain the chromatin in a compact, repressed state. T3 binding results in a conformational shape change of TR, promoting dissociation of corepressor and binding of co-activators and histone acetylases. Subsequent histone acetylation results in chromatin remodelling, facilitating access of the basal transcriptional machinery to the target gene promoter region, with recruitment of RNA polymerase II and subsequent transcriptional activation. (d) The physiological processes mediated by thyroid hormone in humans and the tissue-specific TR-isoform expression patterns. BMR, basal metabolic rate; SHBG, sex hormone binding globulin.

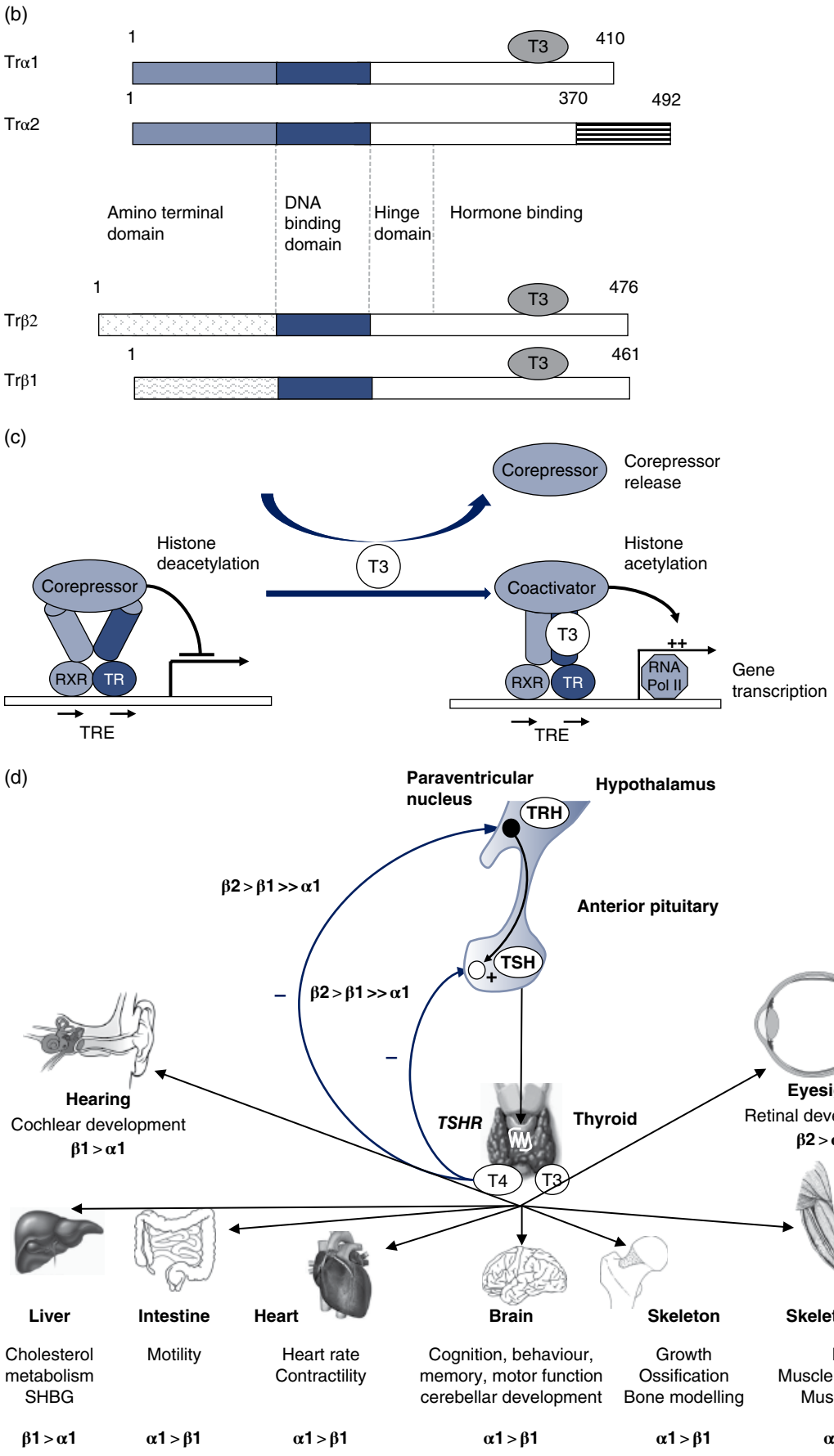

Figure 8.3 (Continued)

Although they are capable of binding some TREs as a homodimer or monomer, heterodimerization with RXR dramatically increases the responsiveness of TR to T3 and transcriptional activation [70].

The domain structure of the thyroid hormone receptor (TR) isoforms is similar to that of other members of the nuclear receptor superfamily. A central, highly conserved DNA-binding domain (DBD) containing two zinc finger motifs interacts with the thyroid hormone response elements (TREs) of TR target genes and may also interact with RXR in the TR-RXR heterodimer. The C-terminal ligand-binding domain (LBD) binds thyroid hormones and is also involved in co-regulator interactions and homo- and heterodimerization of TRs (Figure 8.3b) [71].

Positive Regulation of Genes by Thyroid Hormone

Where genes are positively regulated by thyroid hormones, unliganded TRs mediate basal repression of the gene by binding the TRE together with corepressors and histone deacetylase, modulating local chromatin structure to repress basal transcription. The best characterized corepressors are NCoR and SMRT, which associate with other proteins such as histone deacetylase 3 (HDAC3) and transducin beta-like protein 1 (TBL1) to form large repressor complexes.

Histone deacetylation near the TREs of target genes is important in maintaining chromatin structure in a state that inhibits basal transcription [70]. T3 binding drives a conformational change of the receptor LBD with subsequent co-activator recruitment (e.g. SRC-1, -2, -3) and histone acetylation resulting in relaxation of chromatin and activation of transcription. The crystal structure of the liganded TRα1 has been solved, thereby informing our understanding of the conformational changes that occur when T3 binds to the TR receptor isoforms (Figure 8.3c).

TR LBD structure comprises 12 α-helices surrounding a central hydrophobic pocket. In particular, the C-terminal-most helical segment, helix 12 (H12), contributes to the wall of the ligand-binding cavity and can undergo dramatic shifts in position in response to ligand occupancy. H12 structure and dynamics are paramount for the recognition of co-activators and corepressors that modulate receptor function. In the absence of ligand, H12 is positioned such that it exposes an interface for corepressor binding. Ligand binding perturbs the dynamic equilibrium of H12, which adopts a novel preferential orientation that favours co-activator rather than corepressor recruitment, thus facilitating transcriptional activation of target genes [68, 71–73].

Negative Regulation of Genes by Thyroid Hormone

Many genes, including TRH, TSHα and TSHβ, are negatively regulated by thyroid hormone. The process of negative regulation of transcription by TRs remains poorly understood [68], and postulated mechanisms include recruitment of corepressors instead of co-activators, e.g. Ncor1 during transcriptional repression of murine *Cga* [74]. Additionally, *trans*-repression, where liganded TRs interact with and inhibit the activity of other transcription factors, may be implicated, e.g. GATA2 during TR-mediated repression of *TSHB* [75].

Thyroid Hormone Receptor Function

Isoform Expression

The relative expression levels of the different TR isoforms exhibits tissue-specific differences. Expression of thyroid hormone receptor β2 is largely confined to the pituitary and hypothalamus, where it is the principal mediator of the negative feedback loop regulating circulating thyroid hormone levels, as well as cochlea and retina. TRα1 and the non-hormone binding splice variant TRα2 are expressed in the brain, skeletal muscle, heart, liver, bone, gut, kidneys and lungs. TRβ1 predominates in the liver and kidney [68].

Physiological actions of thyroid hormone are mediated by the TR-dependent transcriptional regulation of target genes in different tissues of the body. Tissue-specific differences in the relative expression levels of the different TR isoforms dictate that specific thyroid hormone-dependent effects are mediated predominantly by one TR isoform (Figure 8.3d).

Thyroid Hormone Effects on Target Tissues

Bone

TRα1, the dominant TR isoform in endochondral and intramembranous bone, is expressed in most skeletal cells, including chondrocytes (reserve and proliferative), bone marrow stromal cells and osteoblasts [67]. Thyroid hormone exerts anabolic effects on the developing skeleton and is crucial for normal bone growth, mineralization and turnover and for the maintenance of bone strength. T3 regulates endochondral ossification and bone elongation mainly by stimulating differentiation of hypertrophic chondrocytes; concomitantly it exerts an inhibitory effect on chondrocyte proliferation [76] and promotes osteoblast differentiation and function [77]. Targets of T3 action in chondrocytes and osteoblasts include growth hormone (GH), IGF-1 and FGF signalling and the Indian hedgehog/parathyroid hormone-related peptide feedback loop [67].

Juvenile hypothyroidism is associated with bone age retardation, epiphyseal dysgenesis (epiphyseal stippling or fragmentation on X-ray), growth retardation and short stature [78–80]. Growth is accelerated and bone age advanced in juvenile thyrotoxicosis but individuals later develop short stature due to premature fusion of the epiphyses [81].

Myocardial Effects of Thyroid Hormone

TRα1 is the most abundant myocardial TR isoform and plays an important role in the regulation of heart rate and contractility [82–84]. Studies in rodent models have demonstrated T3-mediated positive regulation of genes involved both in cardiac contraction and coordination of myocardial electrochemical and mechanical responses (SERCa2, MHC-α, Na^+/K^+-ATPase) [85]. Negative regulation of genes, such as MHC-β, phospholamban and the Na^+/Ca^{2+} exchanger, which have an inhibitory role in cardiac contraction, has also been demonstrated [84].

The electrophysiological effects of thyroid hormone influence both pacemaker and myocyte action potential duration. HCN2, a key driver of the automaticity of pacemaker tissue, is upregulated, thus increasing heart rate. In addition, thyroid status influences expression of voltage-gated K+ channels, e.g. Kv1.5 and the Na^+/K^+-ATPase, which together maintain ion homeostasis and coordinate action potential duration, whereas the Na^+/Ca^{2+} exchanger, which reduces intracellular calcium, is downregulated [84, 86].

In hyperthyroidism, electrophysiological effects of thyroid hormone may manifest as palpitations, tachycardia and atrial fibrillation. Sinus bradycardia, mild diastolic hypertension and narrow pulse pressure in hypothyroidism may be associated with ECG abnormalities such as QT-R interval prolongation, increased QT dispersion, inverted/flat T waves and intraventricular conduction defects [87–90].

Thyroid Hormone Effects on the Gut

Effects of thyroid hormone on gut are thought to be mediated mainly by TRα1, which is expressed both in epithelium and in smooth muscle cells. Appropriate T3 levels are a prerequisite for normal gut motility [91–93] and constipation is a classic symptom of hypothyroidism, reflecting delayed intestinal transit; thyroid hormone excess increases intestinal transit and may be associated with diarrhoea [94, 95].

Hypothyroid constipation may be profound, with case reports describing colonic dilatation and megacolon if untreated [96]. Colonic manometry in hypothyroid individuals demonstrates colonic atony with low amplitude pressures suggesting a reduction in basal neural or humoral control activity with absence of response to muscarinic stimulation in some individuals [97].

Liver

The liver is central in cholesterol metabolism, balancing hepatic cholesterol synthesis and hepatic uptake of plasma lipoproteins from the circulation against the excretion of hepatic cholesterol and bile acids in the bile. TRβ1 is the predominant TR isoform in the liver [98, 99] and modulates these effects [97–101], including stimulation of enzymes regulating lipolysis, lipogenesis and oxidative processes. Overt hypothyroidism is associated with elevated cholesterol and LDL, whereas the reverse occurs in hyperthyroidism [102]. Sex hormone binding globulin (SHBG) is thought to be regulated indirectly by T3-mediated upregulation of HNF-4α gene expression and is known to be elevated in hyperthyroidism, hence its clinical utility as a hepatic marker of thyroid hormone action [103].

Muscle

Skeletal muscle predominantly expresses TRα, and thyroid hormone may have profound effects on skeletal muscle mass, metabolism and contractility; individuals with thyrotoxicosis may rapidly lose muscle mass, and both hypo- and hyperthyroidism may be associated with myopathy [104]. Creatine phosphokinase, an enzyme expressed in skeletal muscle, is well-recognized to be negatively regulated by thyroid hormone, and variable degrees of elevation of CK may be seen in hypothyroidism [105]. Skeletal muscle is also one of the main determinants of basal metabolic rate (BMR), which is influenced by small fluctuations in thyroxine concentrations [106, 107].

Brain

Data from animal models and humans demonstrates that a plethora of neurodevelopmental processes spanning fetal and perinatal time periods is thyroid hormone dependent, including neurogenesis, cell migration and differentiation, synaptogenesis and myelination [108]. Rodent neonatal or fetal hypothyroidism has repeatedly been associated with characteristic cerebellar structural alterations, including stunted Purkinje cell dendrites and delayed granule cell proliferation and migration resulting in a thicker, less mature granule cell layer with fewer Purkinje cell contacts [109]. Oligodendrocyte development and the process of axonal myelination are also thyroid hormone dependent. In the absence of thyroid hormone, myelination is impaired perinatally and in the adult rat [110, 111].

Rodent data demonstrate that 70–80% of total TR isoform expression in the brain comprises TRα1, which is predominantly expressed in the cerebrum and cerebellum. TRβ is the predominant isoform in the hypothalamus, pituitary, cochlea and retina and is also expressed in the cerebellum [68, 112]. Experiments investigating

isoform-specific roles of thyroid hormone receptors in neurological development have demonstrated redundancy in some areas, e.g. cerebellar Purkinje differentiation, which is mediated by TRα1 and TRβ1, whereas other functions are regulated by one isoform only, e.g. cerebellar granule cell migration, which is TRα1-dependent [113].

The key role of thyroid hormone in neurological development is evident from the detrimental effects of thyroid hormone deficiency at different stages of fetal and postnatal development, although the cellular and molecular mechanisms by which thyroid hormone influences these processes are still poorly understood. The fetal brain requires thyroid hormone from early in the first trimester, and inadequate fetal thyroid hormone supply due to maternal deficits during pregnancy and postnatal hypothyroidism due to inadequate endogenous thyroid hormone production both result in neurodevelopmental abnormalities, the nature of which may reflect differential effects of thyroid hormone at different stages of neurodevelopment.

Maternal hypothyroidism has been linked to adverse cognitive and behavioural effects that include autism, impaired visual processing and selective attention and memory problems. MRI imaging of affected children has demonstrated smaller hippocampi and abnormal cortical and corpus callosum morphology. Postnatal thyroid hormone insufficiency, even in the context of appropriately treated CH, has been associated with subtle cognitive deficits, especially in the visuospatial, sensorimotor, memory and attention domains. Visuospatial and sensorimotor deficits may reflect the prenatal duration of hypothyroidism and the severity of CH at the time of diagnosis while language and memory problems correlate with the length of postnatal time preceding treatment initiation [108].

The most severe manifestations of neurodevelopmental impairment are found in endemic cretinism, when severe iodine deficiency during pregnancy results in inadequate thyroid hormone supply to the fetus during early stages of neurological development. In its most profound form, signs may include irreversible motor dysfunction and mental retardation with spastic diplegia, deaf–mutism and extrapyramidal rigidity [113, 114]. Delayed diagnosis of CH, although rare in countries operating a CH screening programme, also results in significant psychomotor impairment and subnormal IQ with growth retardation [115]. Impairment of thyroid hormone biosynthesis in both mother and baby results in more marked neurodevelopmental dysfunction than in CH alone [116].

In adults or older children, hypothyroidism may present with mood change, usually depression, impaired cognitive function and memory or frank confusion or coma [117, 118].

Maturation of Thyroid Hormone Development

In Utero

Transfer of maternal thyroxine to the fetus is restricted by an efficient placental 'barrier' expressing high levels of type 3 deiodinase (DIO3) that regulates the transfer of maternal thyroxine to the fetus by converting maternal T4 to inactive rT3, thus protecting the developing fetus from inappropriately high concentrations of iodothyronines, which are associated with fetal loss [119]. Thyroxine promotes angiogenesis and cell proliferation [70], and excessive concentrations may influence fetal development. However, transplacental transport of maternal thyroid hormone is a prerequisite for normal fetal development, especially during the first trimester when the developing fetal thyroid is unable to secrete T4, and neurodevelopmental consequences may occur if this is inadequate [120, 121].

Evidence in support of maternal T4 transfer despite the placental DIO3 barrier includes the detection of T4 both in fetal coelomic fluids in the first trimester and in cord blood samples from neonates with inability to synthesize T4 due to thyroid agenesis or total thyroid organification defects, thus indicating a maternal T4 source [122, 123]. This mechanism ensures the presence of adequate fetal thyroxine concentrations, even when the fetal thyroid fails to develop normally. The fetus is actually at greater risk from maternal hypothyroidism when there is suboptimal maternal thyroid hormone supply [124].

Fetal transfer of maternal T4 is likely to depend on placental expression of thyroid hormone transporters, binding proteins and DIO3 enzyme activity and, in the case of fetal hypothyroidism, the steep maternal–fetal thyroxine gradient may facilitate fetal acquisition of maternal thyroid hormone [125]. Excessive maternal T4 may overcome the control mechanisms in place and lead to increased risk of miscarriage and lower birth weight. This was demonstrated in a study of euthyroid mothers with raised circulating thyroxine concentrations due to thyroid hormone resistance who exhibited increased fetal loss likely to involve predominantly unaffected fetuses [119].

The fetal thyroid begins to concentrate iodide around 10 weeks of gestation [126], and fetal thyroid hormone production has been shown to increase at a similar gestational timing [127]. The DIO3 inactivation of maternal T4 provides iodide substrate for the fetus, and the placenta is also relatively permeable to iodide.

During pregnancy, fetal thyroid hormone requirements, increased circulating concentrations of thyroid hormone binding proteins (e.g. TBG) and placental DIO3-mediated thyroid hormone degradation necessitate increased maternal thyroid hormone biosynthesis, partially mediated by the actions of human chorionic

gonadotrophin, which is a weak TSHR agonist. Although total T4 concentrations are elevated in pregnancy compared with the non-pregnant state, free T4 concentrations show a transient increase in the first trimester and then progressively decline, and TSH concentrations decrease from approximately week 8 throughout the first half of pregnancy [127]. Fetal TSH concentrations rise throughout the second trimester as the hypothalamic–pituitary axis matures and are consistently higher than maternal concentrations of TSH [127, 128].

Throughout the second and third trimester, fetal total and free T4 and T3 and TBG concentrations progressively increase in parallel to the TSH rise. The concordant rise of T4, T3 and TSH suggests that the fetal pituitary is relatively insensitive to the negative feedback mechanism *in utero*. However, the fetal pituitary will produce TSH in response to exogenous TRH from 25 weeks of gestation [129].

T4 and fT4 concentrations will reach normal adult values by term, but T3 and fT3 concentrations are significantly lower than maternal concentrations; concentrations of reverse T3 peak before 30 weeks and then decline (Figure 8.4). This disparity may be protective, enabling the fetus to maintain low T3 concentrations as a consequence of decreased fetal peripheral conversion of T4 to T3. The high rT3 may reflect enhanced DIO3-mediated deiodination of T3 and/or reduced activity of DIO1, which is responsible for metabolism of rT3 [128].

The Wolff–Chaikoff effect matures towards the end of gestation, and high concentrations of iodine may lead to temporary inhibition of thyroxine production [131]. However, persistently high iodine concentrations result in downregulation of the NIS and reduction of iodide transport into the follicle allowing thyroxine production to restart. Before 36 weeks, this protective mechanism has not matured and high iodine concentrations may induce hypothyroidism. The placenta also remains permeable to antibodies, propylthiouracil (PTU) and methimazole/carbimazole (CBZ), leaving the fetus vulnerable to high maternal intake of antithyroidal medications, maternal TSH receptor antibody (TRAB) and TPO antibodies. Maternal TSH does not cross the placenta.

Neonatal Period

After delivery, there is a surge of TSH and a rise in fT4 concentrations, believed to be stimulated in part due to change in the ambient temperature [132]. The TSH surge commences immediately after delivery, generally peaking in the first 48 hours and settling by day 5 of life [133] (Figure 8.5). This leads to an increase in thyroid hormone production such that fT3 and fT4 concentrations remain relatively higher in infants and fall gradually over the first 6 months of life. The postnatal increase in DIO1 and DIO2 activity and the loss of the placental DIO3 activity also contribute to the fall in rT3 and rise in fT3 [135].

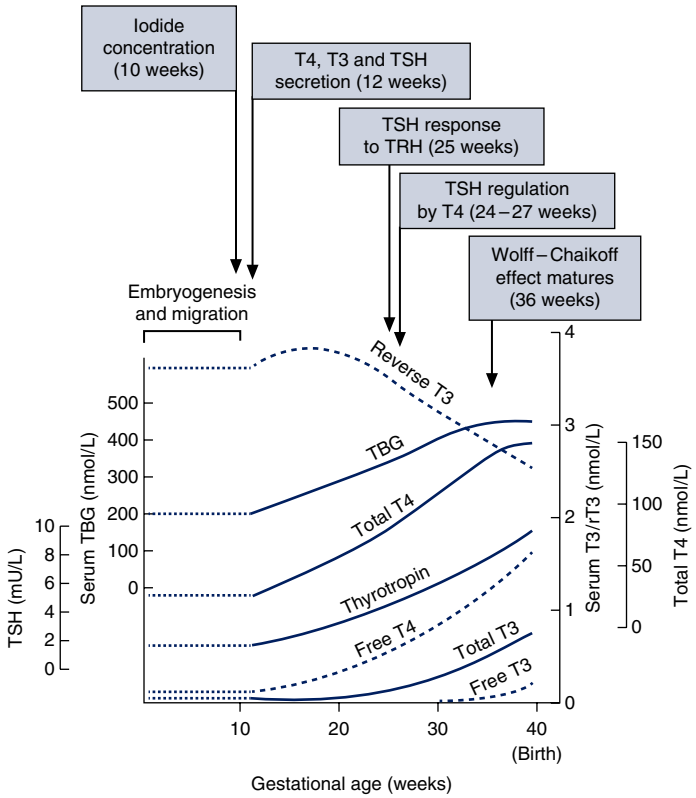

Figure 8.4 Maturation of thyroid gland development and function during gestation. Note that embryogenesis and migration of the thyroid anlage are complete by the end of the first trimester of pregnancy. Serum levels of T4, free T4 and TSH remain low until mid-gestation, however, when the hypothalamicpituitary axis starts to mature and the pituitary does not begin to respond to stimulatory or inhibitory signals until the end of the second trimester. For this reason, during the fi rst half of pregnancy the fetus is dependent on maternal T4. Throughout gestation, the serum level of T3 remains low and the concentration of reverse T3 is high, a consequence of low D1 activity. In the fetus, like in the older child, high iodine exposure results in inhibition of thyroid hormone synthesis (Wolff–Chaikoff effect) but the ability to escape from this inhibition does not mature until late in gestation. For this reason, the premature infant is unusually susceptible to iodine overload. *Source:* From Brown and Larson [130]. Courtesy of Rosalind Brown.

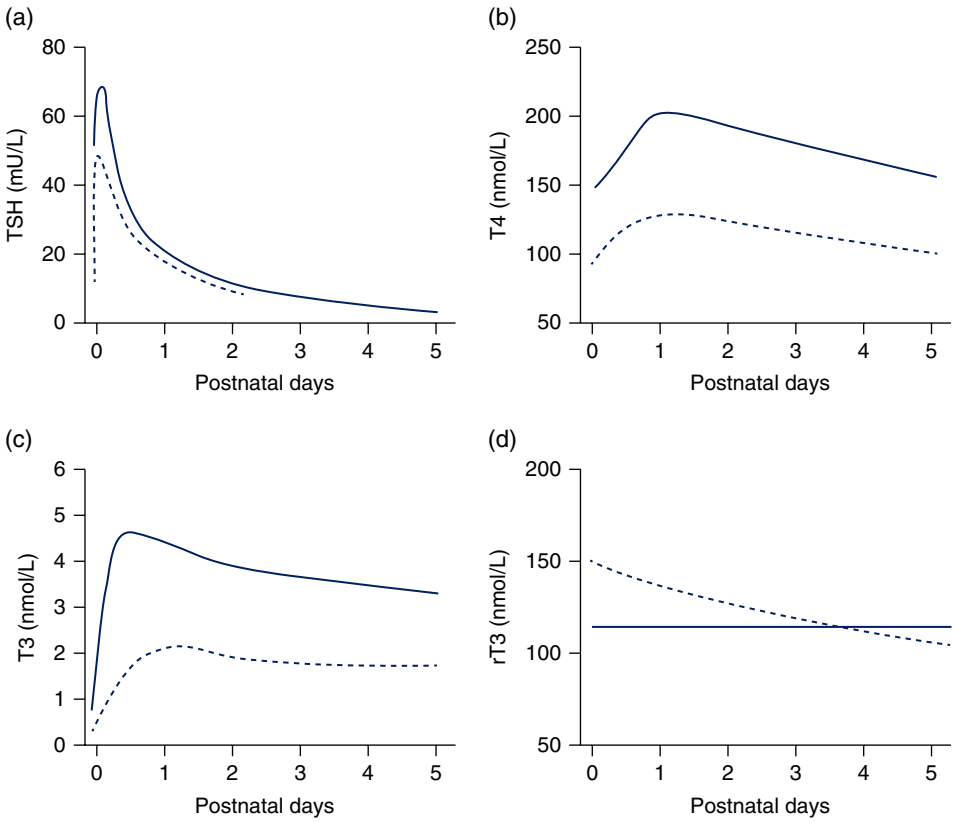

Figure 8.5 Postnatal changes in the serum concentration of TSH, T4, T3 and rT3 in term babies, (continuous line) as compared with premature infants (discontinuous line) in the first week of life. Note that the postnatal surge in TSH is followed by a transient increase in the T4 and T3 concentration in the first few days of life. Changes in premature infants are similar to those seen in term babies but are much less marked. *Source:* From Fisher and Klein [134] as modified by Brown and Larson [130]. Courtesy of Rosalind Brown.

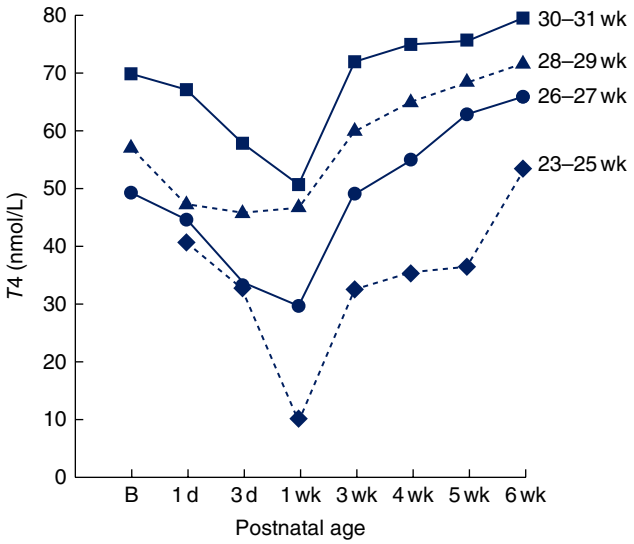

Figure 8.6 Postnatal changes in the serum T4, concentration in premature babies in the first 6 weeks of life. Note that in very premature infants, no postnatal increase in the T4, concentration in the first few days of life is observed. Instead, the T4, concentration decreases with a nadir at 1 week of life. Often the free T4 is not as affected as the total T4 (not pictured). Values subsequently normalize by 3–6 weeks. *Source:* From Mercado et al. [136]. Reproduced with permission of Elsevier.

Prematurity

Premature birth interrupts the changes in thyroid hormone metabolism, which occur over the third trimester, resulting in low T4 and T3 concentrations at delivery in preterm infants, which increase in proportion to increasing gestational age [136] (Figure 8.6). The thyroid hormone concentrations appear to be even lower than those expected *in utero* [137], and the reasons for this are multifactorial, including loss of maternal fT4 in association with an immature TSH-T4 axis, low iodide stores and reduced DIO1 activity.

Additionally, maturation of the hypothalamic–pituitary axis is likely to be delayed in prematurity, and, although the postnatal TSH surge is seen in all gestational ages, it appears to be attenuated in the most premature infants. A natural postnatal nadir in thyroxine concentration occurs at 7 days of life with a subsequent rise thereafter [136, 138, 139].

Some premature infants exhibit subnormal thyroxine concentrations, which are not associated with a rise in TSH. This biochemical phenomenon usually self-resolves and is referred to as transient hypothyroxinaemia of prematurity. The most premature and unwell neonates are likely to have more severe disturbances in thyroid function with markedly subnormal or

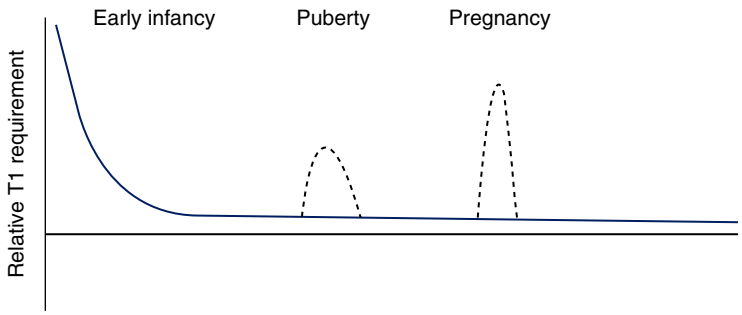

Figure 8.7 Graphical representation of changing thyroid hormone requirements with age or physiological status, high requirements for thyroid hormone at the beginning of life decrease dramatically during the first year of age to reach a plateau, but relative T4 needs may reincrease during accelerated growth in puberty and during pregnancy. *Source:* Figure and legend adapted from Moreno and Visser [141].

undetectable T4 concentrations reported in very low birth weight or the most premature infants, with or without a rise in TSH [136].

Childhood

During childhood, thyroxine requirements decrease with age, suggesting a parallel reduction in thyroid hormone turnover [134]. Circulating thyroid hormone concentrations also decrease with age, highlighting the need for pediatric reference ranges to guide commencement and titration of replacement therapy [140] (Figure 8.7).

Section 2: Clinical Thyroid Disorders

Congenital Hypothyroidism

Classification and Aetiology

CH is the commonest neonatal endocrine condition and has traditionally been classified as TD or dyshormonogenesis (Table 8.1). TD refers to a spectrum of aberrant thyroid gland development, the most common manifestation of which is an abnormally located thyroid gland (thyroid ectopy), accounting for 50–60% of TD. Individuals with thyroid ectopy usually have a round gland lacking the normal bilobed structure, which is located sublingually in the midline due to arrested migration.

An ectopic thyroid is functional but follicular cell mass is decreased compared with a normal thyroid and many affected individuals are or become hypothyroid. In some cases, there are two foci of thyroid tissue (dual ectopy) usually located at the base of the tongue and more distally above the pretracheal position [1, 2, 142], which may be more common than formerly realized [143].

Complete absence of the thyroid (athyreosis) occurs in 20–30% of TD cases; in ~5% of cases, the thyroid gland may be normally located but abnormally small (hypoplasia). Profound thyroid hypoplasia may manifest as apparent athyreosis, where there is no thyroid gland detected by nuclear medicine studies, despite paradoxically measurable thyroglobulin.

Another variant of TD is hemiagenesis, in which individuals, who are usually euthyroid and detected incidentally, lack one lobe of the thyroid gland [1, 2]. 75–85% of CH cases have been attributed to TD with the remaining 20% occurring due to dyshormonogenesis, but recent studies show an increasing incidence of CH together with an increase in cases with a normally located thyroid gland *in situ* (GIS) [144].

Thyroid Dysgenesis

TD is sporadic in >95% cases, but there may be an underlying mutation in *PAX8*, *NKX2-1* or *FOXE1*, which may result in CH with additional congenital abnormalities. Mutations in *TSHR* may cause a spectrum of thyroid dysfunction, including dysgenesis.

PAX8

PAX8 belongs to the paired homeodomain transcription factor family and binds DNA via the conserved paired domain. More than 20 heterozygous, loss-of-function *PAX8* mutations have been reported, which are inherited in an autosomal dominant manner. The majority of reported mutations are missense or nonsense mutations affecting paired domain 2, although promoter region mutations [145, 146] and small deletions have also been described [147]. The biochemical and thyroid morphological phenotype of affected patients is highly variable even within the same kindred, ranging from euthyroidism to severe hypothyroidism. Although thyroid hypoplasia is said to be characteristic, both thyroid agenesis and normal-sized thyroid glands have been reported. PAX8 is also expressed in the nephrogenic mesenchyme and adult kidney and in rare cases *PAX8* mutations may be associated with urogenital tract abnormalities [148–151].

Nkx2-1

Nkx2-1 belongs to the homeodomain-containing transcription factor family, and besides mediating thyroid development, it is expressed in the distal pulmonary epithelium where it plays a role in surfactant production

Table 8.1 A summary of the genetic defects implicated in congenital hypothyroidism.

Gene	Inheritance	Extrathyroidal features	Biochemical features	Radiological features	Additional features
NKX2-1	AD	Neurological (BHC, ~90% cases), respiratory (IRDS, recurrent infections, >50% cases)	Euthyroid–severe CH	GIS–athyreosis	
PAX8	AD	Urogenital tract malformations (rare)	Euthyroid–severe CH	Typically hypoplasia, range from GIS to athyreosis	
FOXE1	AR	Cleft palate, spiky hair (universal), choanal atresia	Severe CH	Athyreosis	
TSHR	AD/AR		Mildly elevated TSH–severe CH	GIS–severe hypoplasia	May be TSH resistant
TG	AR		Euthyroid–severe CH Inappropriately low TG when TSH is elevated	GIS/goitre Normal organification of iodide	May cause fetal goitre
TPO	AR		Usually severe CH	GIS/goitre TIOD	May cause fetal goitre
DUOX2	AD/AR		Mild/transient–severe CH	GIS/goitre PIOD	May be transient or permanent
DUOXA2	AR		Mild/transient CH	GIS/goitre PIOD	May be transient or permanent
Pendrin	AR	Sensorineural hearing loss with EVA	Euthyroid/mild hypothyroidism	GIS/goitre PIOD	
NIS	AR		Euthyroid–severe CH	GIS/goitre Severely impaired thyroid ^{123}I/Tc uptake	May present later in childhood resulting in neurodevelopmental delay
IYD	AR/AD		Euthyroid/severe CH/later-onset hypothyroidism	Goitre Normal organification of iodide	Raised urinary MIT and DIT May present later in childhood resulting in neurodevelopmental delay

AD, autosomal dominant; AR, autosomal recessive; GIS, normally located thyroid gland *in situ*; BHC, benign hereditary chorea; IRDS, infant respiratory distress syndrome; EVA, enlarged vestibular aqueduct. PIOD, partial iodide organification defect; TIOD, total iodide organification defect.

[152]; the ventral forebrain, where it is involved in striated interneuron migration; and the hypothalamic neurons [153, 154]. Heterozygous *NKX2-1* mutations are the commonest transcription factor mutation underlying CH and may cause a triad of brain, lung and thyroid disease.

Penetrance is highly variable, even within the same kindred, and a complete triad is only found in around 50% of patients. Neurological problems affect >90% of cases, usually manifesting as a benign hereditary chorea. Overt or subclinical hypothyroidism occurs in >75% cases, with thyroid morphology ranging from normal (in 55%) to hypoplasia, hemiagenesis or athyreosis. A pulmonary phenotype, most commonly infant respiratory distress syndrome (IRDS), affects 54–78% of cases, and the associated mortality may be up to 16% [155, 156]. A significant number of reported genetic deletions mandate the use of multiple ligation-dependent probe amplification (MLPA) to exclude NKX2-1 deficiency definitively [157], and a high proportion of *de novo* mutations has been reported [155].

FOXE1

FOXE1 belongs to the forkhead/winged-helix transcription factor family. Six loss-of-function *FOXE1* mutations have been described in association with CH. All exhibit recessive inheritance and are located in the forkhead domain. The mutations decrease DNA binding by disrupting either the helical folding of the forkhead domain or the protein–DNA interface.

All affected individuals exhibit athyreosis or severe thyroid hypoplasia, cleft palate and spiky hair. Choanal atresia and bifid epiglottis have also been reported. These extrathyroidal features reflect the expression of

FOXE1 in epiglottis, palate, oesophagus, definitive choanae and hair follicles [158, 159]. Recently, a gain-of-function mutation was detected in association with a similar phenotype [160]. FOXE1 also contains a long polyalanine tract, and the length of this may be associated with risk of TD with a longer tract length being protective [161].

Nkx2–5

Nkx2-5 is a homeodomain-containing transcription factor of the NKX2 family with a well-described role in congenital heart disease but an ambiguous one in TD. Four kindreds have been described in which heterozygous Nkx2–5 mutations have been associated with thyroid ectopy or athyreosis but, in all kindreds, the carrier parents had no morphological or biochemical evidence of CH: one of the reported mutations (p.A119S) has since been described in patients with congenital heart disease but normal thyroid morphology [162, 163]. In a further report of a heterozygous Nkx2–5 mutation in association with a PAX8 promoter mutation, the sister exhibited CH and congenital heart disease but her brother was unaffected despite sharing the same genotype [19].

Evidence for an Undiagnosed Genetic Component in CH

TD most commonly occurs sporadically. There is >90% discordance between monozygotic twins [164] and females are affected more commonly than males, especially in the context of thyroid ectopy [165], which argues against simple Mendelian inheritance. Alternative hypotheses for the aetiology of TD include somatic mutations restricted to the thyroid or epigenetic events. Neither has yet been identified in known genes in sporadic TD, although to date, only small studies have been undertaken [166, 167]. Apparently sporadic cases could also have an oligogenic basis, as has been established for Kallmann syndrome (see Chapter 7). Indeed, mice with heterozygous *TTF1* or *PAX8* mutations are euthyroid, but strain-specific TD occurs in mice with combined partial deficiencies of TTF1 and PAX8 [168]. The latter observation suggests that genetic background may also play an aetiological role.

In apparent contradiction to these data, several lines of evidence, including linkage studies, suggest that genetic mutations may underlie a greater proportion of CH than currently diagnosed; a French national survey of CH cases demonstrated that 2% of TD cases have an affected relative (a figure that is 15-fold greater than predicted by chance alone) and their euthyroid first-degree relatives have more thyroid developmental abnormalities than controls [169–172]. CH occurs more frequently in consanguineous or less genetically diverse populations [173, 174] and is more frequently associated with extrathyroidal developmental malformations [175]. Unexpected occurrence of TD in animal models has yielded novel candidate genes [6].

One way of reconciling these observations is a two-hit mechanism associating a germline predisposing factor with another genetic or epigenetic alteration within the ectopic thyroid tissue itself or in the structures surrounding the thyroid during embryogenesis [176, 177].

TSHR

Loss-of-function mutations in *TSHR* are associated with resistance to TSH and a spectrum of thyroid dysfunction. This may range from a compensated state with elevated TSH concentrations and normal thyroid hormone concentrations and a normal-sized, normally located thyroid gland to overt CH with thyroid hypoplasia or apparent athyreosis.

More than 60 inactivating *TSHR* mutations have been described; the frequency of heterozygous mutations is dependent on the population screened but may be as high as 29% in the context of non-autoimmune hyperthyrotropinaemia [178–180]. The severity of the phenotype is dependent both on the nature of the mutation and the number of mutated *TSHR* alleles.

Patients with complete resistance to TSH and CH require treatment, but it remains controversial whether thyroid hormone supplementation is required in individuals with partial resistance to TSH and compensated euthyroid hyperthyrotropinaemia [180]. Indeed, individuals with *TSHR* mutations may exhibit a reset HPT axis such that supraphysiological fT4 concentrations are required in order to bring the TSH into the normal range. Such treatment may provoke thyrotoxic symptoms, since peripheral sensitivity to thyroid hormone is preserved and individuals have been reported in whom growth and development is normal without treatment and who do not develop pituitary hyperplasia [181].

A recent study achieved long-term follow-up in such cases and concluded that patients with heterozygous mutations, and subclinical hypothyroidism, exhibited a stable compensated state in which hyperthyrotropinaemia was associated with normal thyroid hormone concentrations that did not change over time, thereby obviating the need for thyroid hormone replacement. However, in some patients with homozygous mutations, incompletely compensated subclinical hypothyroidism did evolve, with deterioration of free T4 concentrations into the subnormal range, necessitating treatment with levothyroxine [182].

Dyshormonogenesis

Dyshormonogenesis refers to inadequate thyroid hormone synthesis due to a specific (usually genetic) defect in one of the components of the thyroid hormone

biosynthetic machinery. Clinical, radiological and biochemical investigation, which includes assessment for goitre, severity of CH and presence of extrathyroidal features, together with thyroid imaging and measurement of thyroglobulin may inform the molecular aetiology and enable focussed genetic ascertainment.

In cases where organification of iodide is impaired, a perchlorate discharge test will be positive. This investigation involves the administration of radioiodine (^{123}I) and its quantitation in the thyroid before and after administration of perchlorate, which inhibits further uptake of iodide by NIS. In normal thyroids, >90% of ^{123}I is organified immediately and remains in the thyroid follicle bound to TG but, if organification is impaired, <90% of the thyroidal ^{123}I can be organified and >10% leaks back into the circulation when the second quantification is performed. Severe enzymatic defects cause loss of >90% of the applied ^{123}I dose but enzymatic defects with residual function cause partial iodine organification defined as 10–90% of ^{123}I washout after perchlorate.

CH due to dyshormonogenesis may be permanent or transient. In transient CH, the thyroid hormone biosynthetic defect recovers, usually in the early childhood years. The reason for the recovery may reflect the fact that thyroid hormone biosynthesis is at its peak in the early neonatal period and then declines during early childhood. Since thyroid hormone requirements increase again at puberty and during pregnancy in females, it is important that individuals with transient CH are retested at these times to ensure that thyroid hormone concentrations are adequate.

NIS

Mutations in the NIS are a rare cause of dyshormonogenesis. NIS mediates active transport of iodine across the basolateral membrane of the thyroid follicular cell and enables the thyrocyte to concentrate iodine. Mutations are inherited in a recessive manner and may manifest with severe hypothyroidism or, especially in areas where dietary iodine content is high, with euthyroid goitre. CH due to NIS mutations may not manifest neonatally, and children with normal TSH screening results may develop severe hypothyroidism in infancy with concomitant neurodevelopmental delay [183].

The cardinal radiological features associated with NIS mutations are a blunted or absent radioiodine uptake by the thyroid despite a normally located, usually enlarged gland on ultrasound scan (0–5% vs. the normal range of 10–40%) [184]. NIS is also expressed in gastric parietal cells and salivary glands, which also fail to concentrate iodine in cases harbouring mutations. This feature can be exploited diagnostically, since the ratio of salivary/plasma iodine following injection of radioiodine will be decreased [185].

Pendrin

Pendrin mediates chloride bicarbonate transport in the inner ear where is it essential for maintaining acid–base homeostasis of the endolymphatic fluid. It has a putative role in apical iodine transport in the thyroid follicular cell and is also expressed in the kidney. Individuals with biallelic mutations in pendrin usually exhibit sensorineural hearing impairment and an enlarged vestibular aqueduct (80–100%) with an associated cochlear malformation comprising an incomplete partition of the cochlea (Mondini cochlear malformation) in a subset of cases [186–188].

Pendred syndrome is clinically defined by the association of congenital bilateral sensorineural hearing loss and vestibular dysfunction with diffuse or multinodular goitre resulting from a partial iodide organification defect. The majority of patients remain euthyroid as long as they remain iodide replete. When present, thyroid dysfunction normally presents after the second decade, such that pendrin mutations represent an unusual cause of CH although a frequent cause of familial deafness [184, 189].

TG

Although rare in the general population, thyroglobulin mutations are one of the more common causes of dyshormonogenesis. Mutations are inherited in an autosomal recessive manner and usually associated with moderate–severe CH, although the associated phenotypic spectrum ranges from euthyroidism to severe CH. Goitre, often manifesting in the neonatal period, is a common finding, and affected individuals may exhibit fetal goitre. Diagnostic hallmarks include low thyroglobulin despite elevated TSH and goitre or failure of TG to rise after stimulation with exogenous TSH. Perchlorate discharge testing is normal [38].

TPO

TPO mutations are the main and most common cause of dyshormonogenesis due to a total iodide organification defect [190]. Mutations are inherited in a recessive manner and missense mutations generally cluster around the haem binding catalytic domain. Affected individuals usually exhibit severe often goitrous CH and fetal goitre may also occur. Perchlorate discharge testing usually shows a total iodide organification defect with <10% retained in the thyroid gland [35].

DUOX2

Both monoallelic and biallelic DUOX2 mutations may cause a CH phenotype, usually with partial iodide organification defect on perchlorate discharge testing. DUOX2 mutations are being increasingly described, especially in East Asia [191, 192]. Initially, it was thought that biallelic

mutations were required to cause permanent CH, whereas monoallelic mutations were associated with transient CH [192] but subsequent cases have demonstrated that the distinction is not clear cut and monoallelic mutations may cause permanent subclinical CH [193] while biallelic mutations may cause transient CH [194]. Iodine deficiency is thought to exacerbate the phenotype [184]. Cases harbouring DUOX2 mutations may be missed on neonatal screening since screening TSH measurements may be only marginally elevated; confirmatory venous TSH measurements have been more robustly raised [195].

DUOXA2

DUOXA2 mutations are a rare cause of mild or transient CH, with partial iodide organification defect. Mutations are usually inherited in a recessive manner although monoallelic defects have been described [196, 197].

IYD (Iodotyrosine Deiodinase Deficiency)

The phenomenon of impaired iodide recycling in goitrous individuals is well known but a molecular diagnosis was achieved more recently [198, 199]. *IYD* mutations can cause goitrous hypothyroidism in both monoallelic and biallelic forms, and affected individuals exhibit increased urinary MIT and DIT and rapid thyroidal uptake of iodide with a normal perchlorate discharge test [198, 200, 201]. Hypothyroidism may manifest in childhood rather than in the neonatal period resulting in neurodevelopmental delay [198].

Newborn Screening for Congenital Hypothyroidism

The introduction of newborn screening for the diagnosis of CH has been a major public health success with near elimination of the profound effects of untreated CH (cretinism) in countries where it has been implemented. Before screening, diagnosis was often delayed until infants presented clinically with coarse facies, a large protruding tongue, lethargy, poor feeding, jaundice, umbilical hernia, delayed ossification and hip dysplasia (Figure 8.8), the features of overt hypothyroidism.

Late initiation of treatment resulted in devastating neurodevelopmental consequences [202] including impaired intellectual development and poor mean IQ [203, 204]. The economics of the screening programme are justified by the costs of physical and educational care for the child with neurodevelopmental consequences of CH [205].

The UK screening programme is based on a heel prick capillary blood spot TSH taken at 5–10 days of age. Other screening strategies include capillary blood spot T4 measurements with TSH backup measurements in the lowest T4 centiles. In the Netherlands, screening takes place at day 4–7 of life and the algorithm includes measurement of thyroid binding globulin in addition to TSH for the lowest T4 centiles in order to obtain a proxy measurement of free T4, which cannot be measured on a blood spot. This method detects milder cases of primary CH in addition to central hypothyroidism, which is missed using a primary TSH-based strategy where the TSH may be normal or subnormal [206].

Internationally, some centres use capillary TSH and/or T4 measurements at an earlier age or on cord blood sampling. The timing of sampling determines the screening cut-off value, which is influenced by the postnatal TSH surge and changes in thyroid hormone concentrations in the first week of life. If samples are taken too early, a higher false positive rate is likely.

Increased sensitivity of TSH assays and failure to detect some cases of CH using older screening TSH cut-off values have led to a tendency to decrease the TSH concentration that defines a positive CH screen. In addition, many screening programmes have introduced a borderline concentration range whereby infants are screened a second time a week later. This strategy was introduced to minimize false positive notifications, particularly in cases of a delayed TSH surge.

As well as enabling the identification of children who would otherwise have been missed (false negatives) [207], decreased TSH screening cut-offs have increased the number of infants who are subsequently diagnosed with biochemically milder forms of CH, thereby contributing to the apparent increase in incidence of CH to around 1:2000 [208–210]. An ongoing challenge for screening programmes is to assign appropriate sensitivity and specificity to CH screening based on long-term neurodevelopmental risk, which remains unknown in milder cases of CH.

The screening programme may not identify CH in premature infants with an immature thyroid axis, and, in the UK, all infants born <32 weeks of gestation with a normal TSH screen in the first week of life have a second screening after 4 weeks. Positive and borderline results are referred for venous testing and evaluation.

Evaluation After Referral Through Screening

Biochemistry

Screen positive cases are referred for venous fT4 and TSH measurements and clinical assessment. This should occur within 24 hours of referral in order to commence treatment, where required, as soon as possible.

CH is confirmed when venous fT4 is below the reference range and/or venous TSH is >20 mU/L. Where the fT4 is normal and TSH between 6 and 20 mU/L, repeat testing and clinical judgement is advised [211]. In many cases the TSH will decrease spontaneously into the normal range but, if the fT4 falls or TSH remains persistently raised, treatment with thyroxine is likely to be required.

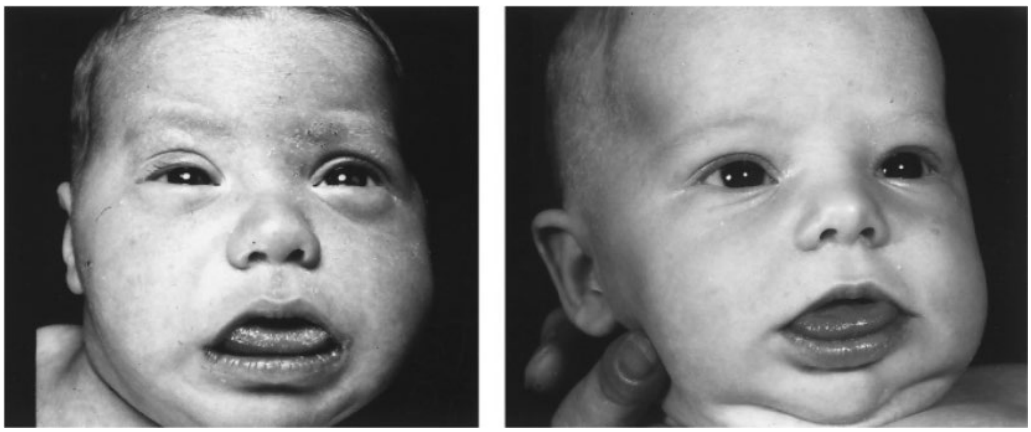

Figure 8.8 Infant with severe untreated congenital hypothyroidism diagnosed clinically before the advent of newborn screening (left), compared with an infant with congenital hypothyroidism identifi ed through newborn screening (right). Note the striking difference in the severity of the clinical features.

The cause and natural history of persistent hyperthyrotropinaemia remain poorly understood. A proportion of neonates judged to be false positive on neonatal screening with an increase in TSH that settled at 2 weeks have been found to have subclinical hypothyroidism in later childhood (9–30%) [212, 213]. Those with TSH concentrations in the normal range appeared to have higher TSH than normal controls [212] and worse neurodevelopmental scores [213]. In an earlier study of 16 infants with hyperthyrotropinaemia and a normally positioned gland, 14 normalized after follow-up 2–7 years later, but 3 children developed a goitre, and 2 had poorer thyroid function. An underlying genetic cause for these findings was not explored [214].

It may be appropriate to measure maternal thyroid function and antibody status when the gland is normally positioned and there is no uptake on technetium scan and/or relevant clinical history, including maternal autoimmune disease and family history. TRAb blocking antibodies can cause severe hypothyroidism and may be missed [215]. The identification of underlying maternal thyroid disease has implications for the infant diagnosis, maternal health and future pregnancies. Some centres routinely measure thyroglobulin, although this is not essential for diagnosis. Low concentrations of thyroglobulin suggest agenesis of the thyroid gland or a thyroglobulin synthesis defect.

Imaging

Imaging of the thyroid gland is important to identify the underlying aetiology of CH. This may have prognostic implications with relevance for counselling parents and planning long-term management. Scintigraphy and/or ultrasound scans will provide information on the size, position and morphology of the gland. Scintigraphy using technetium (^{99m}Tc), which is taken up through the NIS, is cheaper, quicker and more widely used than ^{123}I but an advantage of ^{123}I is its ability to undergo organification, which can then be quantified using the perchlorate discharge test.

Scintigraphy is also dependent on the presence of TSH and optimal images are obtained with higher TSH concentrations. While this requirement should not delay treatment, it should prompt the clinician to scan as soon as possible after diagnosis. Occasionally there will be no uptake on scintigraphy, despite the presence of fT4 in venous sampling before treatment, which may be due to maternal TSHR blocking antibodies or inactivating mutations of the TSHR. Alternatively uptake of ^{123}I or technetium may be impaired by excess iodine intake or mutations in the NIS. In these situations, an ultrasound scan is required to confirm the presence and position of the thyroid gland [216].

Some centres perform X-ray of the knee at diagnosis to assess the presence or absence of the tibial and femoral epiphysis as a marker of severity of intrauterine hypothyroidism [217].

Clinical Assessment

Other congenital anomalies may be associated with CH and should be sought clinically, including cardiac defects (particularly atrial and ventricular septal defects) and urogenital tract abnormalities, in the context of *PAX8* mutations. Infants with CH may have dry or mottled skin, umbilical hernia and jaundice. Macroglossia may be present within the first weeks and the underlying cause of this is not understood. Although it does not always respond immediately to treatment, it resolves as the infant grows. Parents may report that the infant appears more lethargic and sleepy than expected and will often note a rapid change in alertness after commencing treatment.

Treatment

Treatment with levothyroxine should start as early as possible and usually on the day of diagnosis. The dose of thyroxine administered is between 10 and 15 µg/kg. UK guidelines advocate normalization of the TSH within 2 weeks in order to achieve the best neurodevelopmental outcome for the infant, but the fT4 concentrations normalize within a couple of days [218, 219]. Attempts to normalize the TSH more rapidly can lead to symptomatic hyperthyroxinaemia so frequent thyroid function test (TFT) measurements should be performed to adjust L-thyroxine doses in the first few weeks to normalize thyroid function. Infants treated with high-dose thyroxine may require dose reduction within the first week to prevent excessive rises in fT4. Babies with milder thyroid dysfunction often normalize rapidly on the lower doses.

Thyroxine may be administered as crushed tablets or as a commercial solution, both of which may be mixed in a few millilitres of milk to make them more palatable. An advantage of the solution is that accurate dose changes can be made in small increments but the use of thyroxine suspension cannot be recommended since the thyroxine is not fully dissolved and dosing can be variable. The dose should be given using the same preparation at the same time of day [211]. Caution should be exercised if the infant is receiving soy or iron products avoiding concomitant administration if possible or adjusting the thyroxine dose to compensate for impaired absorption [220].

Follow-up

Babies should be reviewed every 1–2 weeks until euthyroidism is achieved and then 1–2 monthly. After 4 months of age, babies require review at least 3 monthly with appropriate titration of thyroxine. The aim of close TFT monitoring is to maintain a TSH, which is unsuppressed and within the normal range [211]. In order to achieve this, the fT4 is likely to be at the top of the normal range. Sometimes the fT4 may be above the normal range and, if the child is clinically euthyroid with normal TSH, this is often appropriate.

In some children with CH, there appears to be some hypothalamic–pituitary resistance to thyroid hormone (RTH) in the first years of life that reduces with age [221]. However, others have reported that the thyroid hormone set point can be permanently altered into adult life [222]. It is unclear if optimal initial treatment at diagnosis with early normalization of thyroid hormone concentrations has a role in 'resetting' the axis.

Despite early thyroxine treatment of children with CH, subtle impairments may still exist, which are related to the timing and duration of hypothyroxinaemia on the fetal and infant brain. Myelination and neuronal development continues after birth, and optimizing thyroid function remains essential, particularly in the first 3 years of life [108, 223]. Deficits in memory and information and auditory processing may manifest in early school life [108]. Episodes of overtreatment with suppressed TSH during the first 6 months of life have been linked to reduced attention and alertness but not to ADHD [224].

There is increasing evidence of hearing impairment in a proportion of treated CH children. This appears to be permanent and to persist into adulthood in some individuals; it is likely to correlate with the severity of the initial presentation [225].

Late Diagnosis of CH

Some cases of CH may be detected late if the infant misses screening or there is an error in the screening process but a small proportion of infants with normal newborn screening present with raised TSH in infancy. TFTs are often performed as part of the prolonged jaundice screen in infancy and in some cases a raised TSH with or without low fT4 will be detected. Hyperthyrotropinaemia with normal fT4 may resolve and treatment depends on clinical discretion.

Reassessment to Establish Permanent or Transient CH

It should become clearer over the first year of life whether an infant is likely to have transient CH. These infants often maintain normal thyroid function without requiring thyroxine dose increments and with normal growth. Doses can be weaned if there is evidence of overtreatment and a trial off thyroxine is recommended at three years of age, particularly for a child on 25 µg or less at this age.

Reassessment at 3 years of age has suggested that about a third of children with a normally placed thyroid gland will have normal thyroid function [226, 227] and there are few data on the long-term outcomes for children with transient CH. Children with transient CH due to dyshormonogenesis, e.g. due to DUOX2 mutations, are theoretically at risk of hypothyroidism in later life when thyroid hormone biosynthesis would normally increase, particularly during pregnancy. Children with confirmed iodine organification defects and transient hypothyroidism have a potential risk of goitre [226] so children with transient CH should have repeat TFT in puberty and pregnancy as a minimum.

Clinical Significance of Genetics

Molecular genetic testing, where available, may be helpful in cases of CH with a normally positioned gland where dyshormonogenesis is suspected or where there is a strong family history or other system involvement. In certain forms of dyshormonogenesis, it highlights the role of iodide in modulating the phenotype and, in some cases, iodide may be an appropriate substitute for levothyroxine therapy. In dyshormonogenesis where

CH may present in childhood following a normal neonatal screening TSH (e.g. due to *NIS* or *IYD* mutations) establishing the genetic aetiology may enable prompt diagnosis in affected siblings to prevent neurodevelopmental delay.

Prematurity

The hypothyroxinaemia of extreme prematurity is usually transient and associated with normal TSH concentrations. Thyroxine concentrations become more markedly subnormal with decreasing gestational age [136]. It is possible that low fT4 concentrations are a biomarker for illness severity or a protective mechanism to reduce metabolic rate. Several studies have suggested that transient hypothyroxinaemia of prematurity (THOP) may be associated with adverse neurodevelopmental outcomes at school age, although a causal relationship has not been established [228–230].

Studies of thyroxine replacement have shown no benefit of levothyroxine treatment for premature infants with hypothyroxinaemia and normal physiological TSH concentrations [231, 232]. In addition, MRI studies of premature infants who were randomized to receive thyroxine therapy suggest no overall difference in white matter changes, although the very immature infants with the lowest T4 measurements had subtle evidence of poorer organization of white matter microstructure [233].

In untreated THOP, there was also no correlation between the fT4 concentrations in premature infants in the first 48 days of life and structural MRI studies at the age of 7 years. It was hypothesized that this was possibly due to resolution of delayed cerebral myelination by this age [234]. In a further study in young adulthood, no association was demonstrated between neurodevelopmental outcomes and THOP [235].

Drugs/Iatrogenic

Iodine preparations may lead to hypothyroidism in the infant, which may be more profound in prematurity as the Wolff–Chaikoff effect does not mature until near term. The use of iodine as a sterilizing agent is no longer routine on most neonatal units due to the permeability of the newborn skin and risk of thyroid uptake. Maternal dietary products (including seaweed) can contain excess iodine and lead to infant hypothyroidism [236]. Similarly, iodine-mimetic drugs such as amiodarone are recognized to cause hypothyroidism in neonates and older children and TFT should be monitored [237]. If amiodarone cannot be stopped, thyroxine may be required during the amiodarone treatment period and titrated appropriately.

Congenital Haemangioma and CH

Up to 10% of infants are born with cutaneous haemangiomas [238]. The natural history is that the haemangiomas can increase during the first year of life and then involute over the next 2–4 years of life. Occasionally these haemangiomas can be extremely large and/or associated with similar haemangiomas in the liver. These large cutaneous and hepatic haemangiomas can express high concentrations of DIO3 resulting in a consumptive hypothyroidism with low fT4 and raised inactive reverse T3. Supportive treatment with endogenous levothyroxine is usually sufficient with weaning as the haemangiomas resolve and the DIO3 activity drops. More severe cases and intensive treatment measures have been described [239].

Fetal and Neonatal Goitre

Fetal goitre is uncommon. It may occur with maternal iodine deficiency as the thyroid enlarges in response to the lack of iodine substrate. In iodine-replete areas of the world, fetal goitre is most likely to arise from dyshormonogenesis or transplacental transfer of maternal antibodies. TRAb stimulating antibodies lead to neonatal thyrotoxicosis and goitre, while inhibitory antibodies and maternal antithyroid drugs (ATD) may lead to hypothyroidism and goitre.

Rarely, fetal goitre can lead to tracheal and oesophageal compression with polyhydramnios and neck hyperextension. There are several published case reports of the administration of intra-amniotic thyroxine to treat fetal goitre, although this remains controversial [240]. There are theoretical risks to the fetus associated with intra-amniotic injection and cordocentesis, and the exact treatment protocol has not been established. In one series, treatment was shown to improve goitre size, although the infants remained hypothyroid at birth [240]. Cordocentesis for fetal thyroid function and thyroglobulin may help to determine the aetiology and monitor interventional treatment [240, 241].

Congenital Hyperthyroidism

Maternal Transfer of Antibodies

Maternal TRAb reaching the fetus transplacentally can have significant effects on the newborn and may remain in the infant circulation for 3–6 months depending on the initial titres. TRAb antibodies may be inhibitory or stimulatory, and sometimes both types coexist such that differential titres and clearance of the two antibodies can lead to a delayed presentation of thyroid dysfunction [242].

Although prediction of susceptible infants can be achieved by monitoring antibody titres in the mother

during pregnancy, such investigation may not be undertaken in euthyroid mothers with a past medical history of Graves' disease (GD) treated with surgery or radioactive iodine. In these women, antibodies can remain or recur during pregnancy and cross the placenta to the fetus.

Clinical Presentation

Neonatal thyrotoxicosis can be life-threatening. Affected infants present with tachycardia and jitteriness, with poor subcutaneous fat tissue, voracious feeding, poor weight gain, lid retraction and goitre. Without the knowledge of a history of maternal thyrotoxicosis, the diagnosis can be missed and treated as sepsis [243]. The tachycardia may progress to arrhythmia and cardiac failure, and the mortality rate is up to 20% [244].

Treatment

Treatment with beta blockers and ATD (usually methimazole or CBZ, which is converted to methimazole) is often required and may need to be continued for several months with close monitoring and dose reduction as the antibodies clear. Liver dysfunction can result from the thyrotoxicosis *per se* and should be checked before starting ATD. A maculopapular rash can occur and may be missed due to its non-specific appearance. Neutropenia may also occur as a side effect of therapy and full blood count checks with repeat TFTs are recommended.

Treatment with beta blockers can normally cease once euthyroid status is achieved, although methimazole may be required for several months as the TRAb antibodies clear. Methimazole doses can be reduced in conjunction with TFTs and TRAb antibody monitoring.

TSH Receptor Mutation

Heterozygous activating TSHR mutations result in familial non-autoimmune hyperthyroidism (FNAH) inherited in an autosomal dominant manner from an affected parent or in sporadic congenital non-autoimmune hyperthyroidism (SCNAH) in the case of a *de novo* mutation affecting all cells of the thyroid gland. The age at diagnosis of thyrotoxicosis may be variable but SCNAH tends to be associated with earlier presentation (neonatally 11 months) and more severe disease.

In FNAH, hyperthyroidism may be mild/subclinical or severe, with the age of manifestation ranging from the neonatal period to late adulthood although intra-familial differences in the severity and age at presentation of the thyrotoxicosis may be marked. Goitre, usually diffuse in childhood and multinodular in later life, is present in >50% cases and early presentation may be associated with advanced bone age. Multiple relapses after ATD therapy or inadequate doses of radioiodine are frequent when the condition is confused with GD.

Most infants with SCNAH are born prematurely and may have IUGR: fetal or neonatal hyperthyroidism may be severe, necessitating medical treatment initiation in an intensive care unit (ICU). Prompt and effective control of hyperthyroidism is essential to avoid complications that include craniosynostosis, advanced bone age and psychomotor retardation; goitre and proptosis may also be present. Thyroidectomy or ablative radiotherapy are required for permanent cure of both SCNAH and FNAH [245, 246].

McCune–Albright Syndrome

McCune–Albright syndrome results from an activating mutation of the G α subunit, which results in excess stimulation of adenylyl cyclase leading to increased intracellular cAMP concentrations. The somatic mutations can lead to endocrinopathies including Cushing syndrome, precocious puberty, GH excess and thyrotoxicosis. It is associated with characteristic large café au lait skin pigmentation and polyostotic fibrous dysplasia.

Thyroid hyperfunction is associated with nodular goitre at any age, including in the neonatal period [247]. In addition to the excessive production of fT4 without TSH drive, there appears to be overactivity of intrathyroidal DIO1 and DIO2 resulting in the conversion of T4 to T3 and a high T3/T4 ratio [248]. Treatment with methimazole is usually effective, although thyroidectomy is curative.

Acquired Thyroid Disorders

Autoimmune Thyroid Disease (AITD)

The thyroid gland is susceptible to autoimmune disease with the development of autoantibodies against TPO, thyroglobulin and TSHR antigens. Stimulating antibodies to the TSHR are associated with GD, while antibodies to TPO and TG are generally associated with a chronic lymphocytic thyroiditis (Hashimoto's thyroiditis). However there is considerable overlap between these two conditions with reports of Hashimoto's thyroiditis preceding GD [249–251] and up to 70% of patients with GD also have TPO or TG antibodies present [252]. TSHR antibodies may be inhibitory as well as stimulatory, which can further confuse the clinical picture.

Polymorphisms in the genes encoding TG and the TSHR and a number of immune regulation genes are responsible for 70% of autoimmune thyroid disease (AITD) and environmental exposures, including iodine status and infection, are presumed to account for the rest [252]. Smoking appears to increase the risk of Graves' while protecting against Hashimoto's [252].

AITD is associated with polymorphisms in a range of thyroid-specific genes and immunoregulatory susceptibility genes, which are ordinarily responsible for the development of self-tolerance. The majority of the identified genes are not specific to either Graves' or Hashimoto's and include FOXP3, CTLA-4, PTPN22, FCRL3 and several HLA subtypes. Variants in TSHR, CD40 and CD25 appear to confer an increased risk of Graves' [253]. Polymorphisms in HLA-DR3 confer the greatest risk and are linked to a 2–6 times increased risk of developing GD or autoimmune thyroiditis in Caucasians while HLA-DR4 and HLA-DR5 are associated with an increased risk of goitre [254].

This high genetic susceptibility rate may account for the strong family history of AITD, with 70% heritability in twins [255], and the association with other autoimmune conditions such as type 1 diabetes, coeliac disease, vitiligo and alopecia [256]. AITD also has an increased prevalence in conditions of aneuploidy including Turner, Down and Klinefelter syndromes [251, 257–259].

Juvenile AITD has an increasing prevalence with age and is therefore most common in the pubertal age group. It has been demonstrated that the incidence of pediatric thyrotoxicosis appears to be increasing over time in common with other childhood-onset autoimmune conditions, such as type 1 diabetes and coeliac disease [256]. The sex ratio is similar in those under 4 years of age whereas an increasing female/male ratio is observed with increasing age [256].

Hashimoto's Thyroiditis

Hashimoto's thyroiditis is the most common acquired thyroid disease of childhood. The onset of the autoimmune process is marked by the infiltration of lymphocytes into the thyroid gland with subsequent cell cytotoxicity, apoptosis and antibody production. Cytokine production by the infiltrating lymphocytes further fuels the inflammation and destruction of the thyroid architecture [260]. Destruction of thyroid follicular cells can lead to diffuse enlargement of the thyroid, often with a palpable or visible goitre and a typical hypoechoic image on ultrasonography.

Clinical Features of Hypothyroidism

The presentation of hypothyroidism may be of slow onset, delaying diagnosis. Children may be noted to sleep more than expected and complain of a lack of energy; school work and cognition may be impaired but the children are generally quiet and non-disruptive in school so this is rarely a cause for complaint.

Physical effects include dry skin, constipation and poor tolerance to cold. Occasionally hair loss or thinning is noted. With time, growth falters with delay in skeletal and dental maturation and puberty. Body proportions may remain immature and the weight-for-height ratio will increase. Severe hypothyroidism is associated with slipped upper femoral epiphysis [261].

Chronic hypothyroidism can lead to a myxoedematous facies, bradycardia, muscle pseudohypertrophy and slow relaxing deep tendon reflexes (Figure 8.9) [261]. Chronic

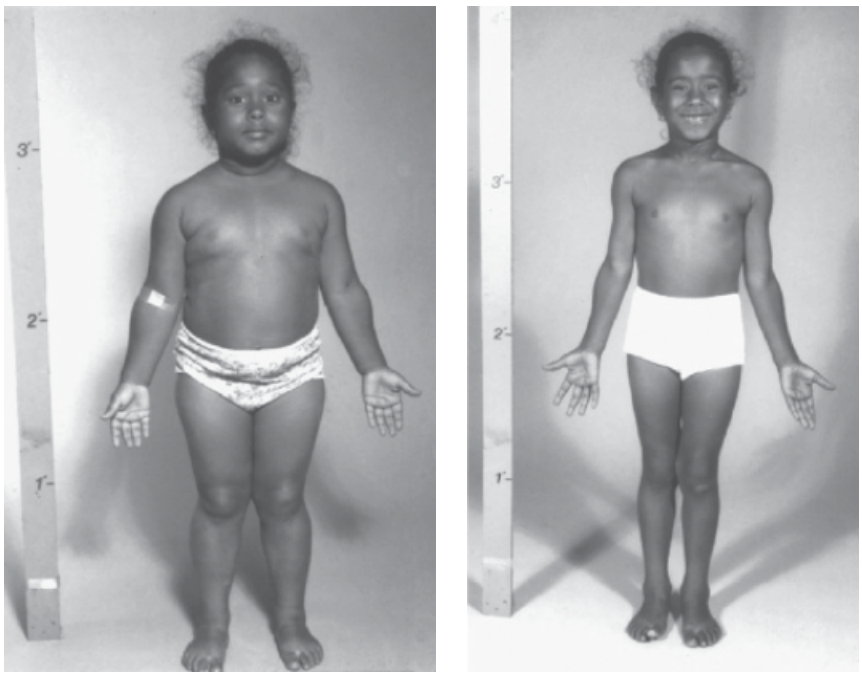

Figure 8.9 Ten-year-old female with severe primary hypothyroidism caused by primary myxedema before (a) and after (b) treatment. Presenting complaint was poor growth. Note the dull facies, relative obesity and immature body proportions before treatment. At age 10 years she had not lost a deciduous tooth. After treatment was initiated she lost six teeth in 10 months and had striking catch-up growth. Bone age was 5 years at a chronologic age of 10 years. TSH receptor blocking antibodies were negative.

elevation of TSH can lead to an FSH effect with testicular enlargement in males and ovarian cysts in females and may progress to a form of pseudo-puberty (Van Wyk–Grumbach syndrome) [262]. Irregular menses may be noted in girls.

The goitre associated with Hashimoto's can be large and is usually symmetrical but asymmetry does occur and the surface may not be smooth.

Diagnosis

Hypothyroidism secondary to Hashimoto's thyroiditis is confirmed by a raised TSH, low fT4, the presence of TPO or TG antibodies and/or a typical ultrasound appearance but, in Hashimoto's thyroiditis, goitre is often the only presenting feature and 50% of children at diagnosis are euthyroid [263]. Younger children and those with Down or Turner syndrome are more likely to have biochemical abnormalities at presentation [263]. Other cases may be detected on incidental biochemical testing or screening in the presence of other autoimmune conditions: diagnosis is then based on the presence of antibodies and the ultrasound appearance.

Management

When a child presents with overt biochemical hypothyroidism and TPO or TG antibodies, further investigations are not usually required and treatment with levothyroxine should be initiated at a low dose and increased slowly to therapeutic doses around 100 µg/m^2/24 h, although the dose needs to be titrated using TFTs. Treatment is lifelong and regular follow-up of growth and dose adjustment is required.

Biochemical changes associated with commencement of levothyroxine treatment can lead to headache, insomnia and deteriorating school performance. Parents should be advised to inform school staff of a predicted change in concentration and behaviour as the child becomes more alert with treatment. A rarer complication is pseudotumor cerebri, which can present with severe, persistent headache associated with rapid changes in thyroid hormone after treatment [264].

In up to 50% of children presenting with Hashimoto's thyroiditis, there is subclinical hypothyroidism with normal fT4 and a TSH concentration that is raised above the normal reference range but below 10 mU/L. This may not require treatment [265]. There is little evidence of benefit of levothyroxine treatment in children with euthyroid goitre secondary to Hashimoto's thyroiditis [266]. In a retrospective cohort that studied children with Hashimoto's thyroiditis over a 32 year period, TSH concentrations fluctuated and a total of 52.5% of children remained or became euthyroid after 5 years [267].

Hashitoxicosis

A transient hyperthyroid phase, termed Hashitoxicosis, can occur due to the release of stored T4 and T3 as the thyroid is damaged. This may last for weeks to months [268] and, when present, symptoms can usually be managed with beta blockers. It has been suggested that the severity of presentation and duration of hyperthyroidism may be related to the TPO antibody concentrations [269] but, if TRAb antibodies are also present, the persistent TSH stimulation can lead to a more prolonged and clinically significant phase. Thyroid scintigraphy has been used to determine the underlying pathology with increased uptake due to TSHR stimulation (as seen in GD) and a reduced uptake in Hashitoxicosis [270].

Treatment with methimazole may be effective, although in most cases the Hashitoxicosis will resolve and the child is likely subsequently to develop hypothyroidism. Close monitoring of thyroid function is essential.

Graves' Disease

GD is the most common cause of hyperthyroidism in children [256]. The incidence is between 1 and 3/100,000 [256, 271] and it is seen most often in girls in the 10–15 year age group. It is caused by the presence of TRAb.

TRAb antibodies act on the TSHR, leading to hyperstimulation of the thyroid gland and follicular growth. Subsequent infiltration by lymphocytes and B-cell dysregulation lead to diffuse thyroid enlargement and a palpable goitre [252, 272]. Imaging is not essential, although a thyroid USS with or without fine needle aspiration (FNA) is recommended if there is evidence of a nodule or asymmetry of the thyroid gland. Thyroid carcinoma is a rare association with GD in childhood [272].

Clinical Features of Hyperthyroidism

Presentation is often delayed, particularly in the younger child. Restlessness, agitation and poor concentration may be misdiagnosed as attention deficit hyperactivity disorder (ADHD) and deteriorating school performance is often reported. Other symptoms include headache, tachycardia, difficulty in sleeping, low energy, heat intolerance, muscle fatigue and poor weight gain despite an increased appetite. If undiagnosed, hyperthyroidism can lead to an increase in growth velocity, weight loss and advance of bone age. This is seen most often in the prepubertal child and this group characteristically presents with higher fT4, fT3 and TRAb concentrations [272]. Growth can be preserved with prompt treatment [273]. Puberty may be delayed and secondary amenorrhoea may occur.

Physical signs include tachycardia with a wide pulse pressure, tremor, fidgetiness and sweatiness. The goitre,

when present, is usually smooth, symmetrical and diffuse and a bruit may be found on auscultation.

Thyroid eye disease occurs in children and the incidence increases with age, TRAb concentrations and exposure to tobacco smoke. Lid retraction or 'staring' appears to be correlated directly to hormone concentrations and resolves when a euthyroid status is achieved. Proptosis and lid lag are the most common eye manifestations in children. The underlying autoimmune process leads to increased fibroblast activity with glycosaminoglycan production within the orbital fat tissue producing an osmotic gradient with oedema and swelling that results in proptosis [274]. Swelling of the ocular muscles, strabismus and optic neuropathy are rare [274, 275], although the need for orbital decompression has been reported [276]. Corneal punctuate staining may occur and dry eyes with redness and itching may be helped with artificial tears. Glucocorticoid treatment may be necessary in the most severe cases.

Diagnosis

Hyperthyroidism is characterized by a suppressed TSH with a raised fT4 and fT3. In some cases, a raised fT3 can be seen with a near-normal fT4 (T3 toxicosis). A clear history and thyroid antibodies are helpful to confirm the diagnosis and to differentiate Graves' from Hashitoxicosis, although TPO and TRAb antibodies can coexist and, in some cases, antibodies are not initially detected. Thyroid ultrasound would be indicated if there was concern over a palpable nodule.

Management

Initial management is aimed at achieving euthyroid status and propranolol is a useful adjunct at presentation to provide symptomatic relief until this is achieved. First-line treatment is usually with ATD, which are effective, but remission rates in children are estimated to be around 30–40%, which is significantly lower than in the adult population.

Factors associated with poorer remission rates include younger age, higher fT4, fT3 concentrations and TRAb antibody titres at diagnosis [277]. Only 25% of patients are likely to achieve remission after 2 years [278] and cumulative remission rates increase up to 8 years of antithyroid medication but, when treatment is discontinued, over a third of young people relapse [279].

ATD have a side effect profile that deters long-term use and definitive treatment with radioactive iodine and surgery are often required. The hesitancy to move to definitive treatment in childhood is partly due to the risks perceived with surgery and radioactive iodine treatment. Lifelong treatment with levothyroxine will be required and adherence is particularly important in childhood and puberty.

Overall duration of ATD treatment tends to be longer in children than adults, which may be dependent on access to an experienced surgeon or for the need to wait until the child is of an age suitable for radioactive iodine.

Radioactive iodine is also not recommended in cases of thyroid eye disease, which can potentially worsen after radioiodine due to a rise in TRAb antibodies after treatment [280].

Antithyroid Drugs (ATD)

ATD reduce synthesis of T4 and T3 by inhibiting organification of iodide and coupling of iodothyronines [281]. They do not affect the release of stored thyroid hormones or treat the underlying autoimmune process and do not influence the time taken for remission.

First-line therapy is usually methimazole that can be given as a single or divided doses. fT4 and fT3 fall as soon as treatment is commenced and may take up to a month to normalize. The dose of methimazole should be reduced at this point to prevent overtreatment. The TSH can remain suppressed for many weeks after this. The methimazole dose is then titrated to maintain normal TFT. Some clinicians prefer to block the thyroid gland completely and replace with levothyroxine. Both are effective treatment options, although the latter is likely to require higher ATD doses and thus an increased risk of side effects [282].

Side effects of methimazole include agranulocytosis, Stevens–Johnson syndrome and, more commonly, skin rashes. Moderate side effects were estimated to occur in up to 11% of children in one study with 90% of adverse reactions manifesting in the first year of therapy [283]. Agranulocytosis is associated with dose of treatment and usually occurs within the first 3 months [283, 284]. Families should be warned to seek medical attention if a child treated with methimazole develops a severe sore throat or mouth ulcers. Methimazole is also known to be teratogenic leading to cutis aplasia and choanal atresia so PTU is the preferred treatment option in pregnancy.

PTU inhibits peripheral conversion of T4 to T3, which may theoretically improve effectiveness in severe hyperthyroidism, but it is no longer used as first-line therapy due to an association with irreversible liver failure, which warranted liver transplant in an estimated 1 in 2000 children in the USA [285]. In addition, the estimated number of children with reversible hepatotoxicity from PTU treatment was 10 times greater than this (1 in 200) [285]. Monitoring liver function was not helpful since the deterioration in liver function was rapid and irreversible. PTU is therefore reserved for second-line treatment in instances of failure of methimazole therapy or side effects [286].

Thyroidectomy

Surgery should be performed only by experienced surgeons with evidence of a high throughput of pediatric thyroidectomies. This minimizes the risk of severe complications including laryngeal nerve damage and/or hypoparathyroidism. Transient hypocalcaemia is a common side effect [287–289] and the cosmetic appearance of a thyroidectomy scar may also deter some young people. Thyroid surgery is recommended for patients with thyroid eye disease or large thyroid glands in preference to radioactive iodine [290].

It is important that the child is euthyroid before surgery and, if this cannot be achieved with ATD, a short treatment with Lugol's iodine (potassium iodide) is recommended to block the thyroid gland through the Wolff–Chaikoff effect. It has the additional advantage of reducing vascularity seen with large goitres.

Radioactive Iodine

Radioactive iodine is considered safe and effective for those over the age of 10 years [291]. Some centres use it in younger children, although concerns exist about the risk of malignancy and the practicalities of isolating the child from other young family members and peers for the weeks after treatment. ^{131}I radioactivity persists in the thyroid for several days and is excreted in saliva, urine and stool. Treated children must not be in contact with others who would be placed at risk to this secondary exposure.

The concern around risk of malignancy after ^{131}I therapy has arisen after studies of individuals exposed to thyroid irradiation as children reported an increased incidence of thyroid cancer [292]. This risk appeared to be greatest for the youngest children, particularly those under 5 years of age, and with low doses of irradiation. Lower-dose ^{131}I treatment has also been associated with later recurrence of GD and hyperparathyroidism [291]. There is no evidence of an increase in other cancers after childhood ^{131}I for Graves' [291] or genetic risk to offspring of children previously treated with ^{131}I [287].

Given the evidence to date, it is recommended that, when radioactive iodine is administered to young people, it should be with the aim of thyroid ablation. Some centres use a fixed dose of 15 mCi ^{131}I [293] or treat with a dose of >150 μCi ^{131}I per g of thyroid tissue. It should be avoided in children under 5 years of age and, between the ages of 5 and 10 years, the treatment dose should be <10 mCi [286]. It is recommended that euthyroid status is achieved before treatment to avoid the increased risk of thyroid storm [286, 294].

After treatment there may be a transient rise in fT4 concentrations and hypothyroidism is usually achieved after 2–3 months in 95% of cases [295]. A second course of ^{131}I may be indicated if the child remains hyperthyroid.

Thyroid Storm

Uncontrolled thyrotoxicosis can lead to this potentially life-threatening condition. It is fortunately rare with a pediatric incidence of 0.1–3/100,000 [296]. It is a multisystem disorder driven by the high circulating thyroid hormones resulting in fever, tachycardia, arrhythmia, deranged hepatic function and confusion, which in turn can lead to shock, cardiac failure, disseminated intravascular coagulation, coma and death [286, 294, 297].

Thyroid storm may be precipitated by surgery or radioactive iodine, particularly if these are performed before euthyroid status has been achieved [286]. It is unclear if radioactive iodine treatment *per se* can cause thyroid storm or if the storm is associated with prior biochemical hyperthyroidism from withdrawal of ATD or failure to achieve euthyroid status before treatment.

The severity of the condition is directly related to the biochemical status and rapid treatment is required [297]. ATD may be used, although the use of potassium iodide will block the thyroid gland hormone production more rapidly. Supportive therapy, beta blockers and steroids may also be required.

Thyrotoxic Periodic Paralysis

This is an extremely rare complication most likely to present in adult men of Asian ethnicity, although it has been reported in adolescence [298]. The paralysis is secondary to acute hypokalaemia: it is thought that thyroid hormones may increase the activity of the Na^+/K^+-ATPase responsible for transport of these two ions across cell membranes, which leads to an intracellular shift of potassium and a low circulating intravascular potassium concentration [299]. Mutations in the gene encoding Kir2.6 have been identified in some patients. This is a potassium inward rectifying channel and it is suggested that loss of function of this channel prevents efflux of potassium and maintenance of normal potassium concentrations across cell membranes [300].

It occurs on a background of thyrotoxicosis and is more common in men, particularly after stressful situations, exercise and carbohydrate intake. These factors are associated with catecholamines, thyrotoxic concentrations of fT4 and fT3, insulin and androgen concentrations, which all contribute to the overactivity of the Na^+/K^+-ATPase and act as triggers for the paralysis. Thyrotoxic periodic paralysis is a recurrent condition affecting proximal muscles with clinical severity ranging from mild weakness to flaccid paralysis. The hypokalaemia may also lead to life-threatening arrhythmia and close cardiac monitoring is required [299].

Treatment includes the use of beta blockers and cautious administration of potassium in the acute phase. ATD administration should be commenced and the condition completely remits once euthyroid status is achieved.

Other Forms of Thyroiditis

Acute Infectious Thyroiditis

This inflammation of the thyroid gland is caused by infection, which is commonly bacterial, although fungal and parasitic infections are reported [301]. In children it is often associated with a fistula from the pyriform sinus, a remnant arising from the path of migration of the ultimobranchial body from the fifth pharyngeal pouch during embryonic development. It is generally limited to the left side since the right ultimobranchial body is often absent. Infectious thyroiditis is also seen when a child is immunocompromised and, in these instances, can be associated with less common infectious agents.

Clinically the child will present with a painful anterior neck often leading to a fixed head position associated with fever, lymphadenopathy and systemic signs of infection. An intrathyroidal abscess can form and a discharging cervical fistula may be seen [302]. TFTs are usually normal, although hyperthyroidism and hypothyroidism have been reported [301].

USS (or CT) may detect areas of infection and the presence of a fistula and FNA can help identify the infectious agent and guide treatment [303]. If a fistula between the thyroid and sinus is suspected and not seen, a barium swallow may help further assessment. After initial antibiotic treatment and resolution of the thyroiditis, removal of the fistula with or without hemithyroidectomy is recommended to prevent recurrence [301, 302].

Subacute (De Quervain's) Thyroiditis

The cause is unknown, although often assumed to be viral, and is rare in children [304]. It has been reported to occur within families and there is an association with HLA-BW35 [301, 305]. It causes smooth, painful enlargement of the thyroid associated with fever, lethargy and myalgia. An initial hyperthyroid phase is seen in half of cases. A transient hypothyroid phase can then occur in some patients before resolution of the illness [301, 304].

A markedly raised ESR and thyroglobulin are common and thyroid antibodies are not usually present. Radioactive iodine uptake is likely to be low and a scan is not routinely indicated. Treatment is supportive with pain relief, non-steroid agents and prednisolone in severe cases.

Euthyroid Goitre

Iodine deficiency and dyshormonogenesis may present initially with euthyroid goitre and simple goitre without thyroid dysfunction; the presence of antibodies may be detected incidentally and can be associated with puberty [306]. One large retrospective study suggested that the presence of thyroid autoantibodies in euthyroid goitre is associated with up to 20% risk of evolving thyroid dysfunction [307] and therefore children presenting with goitre should be evaluated for antibody titres in addition to TFTs. Children with evidence of Hashimoto's thyroiditis have a high risk of deteriorating thyroid function compared with those with simple idiopathic subclinical hypothyroidism [308].

Thyroid Nodule

Thyroid nodules are rare in children and must raise a high index of suspicion for malignancy because up to 18% of single nodules are malignant [309]. Thyroid scintigraphy may determine if a nodule is hot (functioning) or cold (no uptake) but does not exclude thyroid carcinoma. A USS-guided FNA can be helpful and diagnostic in up to 90% of cases [310]. Follow-up of a benign nodule is required to monitor growth and progression and in many cases will ultimately result in surgical resection with hemithyroidectomy.

Iodine Status

Iodine deficiency has been recognized to be a leading cause of hypothyroidism and goitre for decades. Targeted programmes of iodine supplementation in geographical areas of known iodine deficiency have been successful with improvement in thyroid status and incidence of goitre. In the UK, iodine status and endemic goitre initially improved through serendipity and changes in dairy farming practice [311] but the UK population has been dependent on dairy products to maintain iodine status and further changes to farming methods and dietary habits have led to a decline in population iodine status. A recent analysis of UK schoolgirls confirmed this phenomenon with a high incidence of mild–moderate iodine deficiency [312].

Individual quantification of iodine concentration is difficult due to fluctuating concentrations. Studies are therefore focussed on population iodine status and there is evidence that improving childhood iodine status can have significant effects on intellectual outcomes and growth within the population treated, probably as a direct result of improved thyroid function [313, 314]. The same mechanism explains why children with subclinical hypothyroidism secondary to iodine deficiency

can demonstrate an improvement in metabolic profiles with iodine treatment [315].

Iodine concentrations in pregnancy directly affect the provision of substrate to the fetal thyroid and therefore influence thyroxine production. Low maternal iodine concentrations may adversely affect the long-term neurodevelopmental outcomes of the offspring [316]. The economic benefits of iodine supplementation have been costed [317] but it is unclear whether iodine supplementation can improve outcome and there is a suggestion that it may even be detrimental [318]. It is likely to be of greater benefit to the fetus if the mother is iodine replete before pregnancy rather than changing the fetal environment with iodine supplementation once pregnancy is already established.

Changes in iodine status in populations have been shown to lead to a temporary increase in the incidence of autoimmunity, including GD [319] and subclinical hypothyroidism. This population effect is thought to be transient and overall the benefits of treating iodine insufficiency outweigh an increase in temporary thyroid dysfunction [320].

Section 3: Diagnostic Pitfalls

Normal Thyroid Hormone with Elevated TSH

Subclinical Hypothyroidism

This is defined as normal thyroid hormone concentrations with a TSH greater than the top of the reference range but <10 mU/L. In some cases it may be an early manifestation of AITD, although antibodies will often be negative. Follow-up studies suggest that the majority of antibody negative (idiopathic) cases will normalize with time [321, 322] and thyroid function is unlikely to worsen.

Subclinical hypothyroidism is seen commonly in children with trisomy 21 (with and without positive antibodies) and in this situation is thought to be due to poor regulation of the hypothalamic–pituitary axis [323]. With increasing age, children with trisomy 21 have an increased risk of developing AITD [322].

In children over the age of 3 years, the myelination and structural brain development is complete. There is no evidence that subclinical hypothyroidism in this older age group is associated with poorer neurodevelopmental outcomes, growth or bone health [265].

In asymptomatic children, thyroid function and thyroid antibodies should be monitored. It is unclear whether treatment confers benefit and there is insufficient evidence to recommend this [265]. However, long-term elevation of TSH is associated with poorer long-term metabolic markers and cardiovascular health [321].

Obesity

Routine biochemical testing of obese children suggests that raised TSH with normal fT4 and fT3 is common. It may be present in up to 20% of obese children and the TSH has been shown to normalize with weight loss, suggesting that the thyroid dysfunction is a consequence of the obesity and not an underlying cause [324]. It has been hypothesized that obesity leads to inflammatory cytokine release inducing lymphocytic thyroid infiltration, which results in abnormal thyroid USS findings [260]. Alternatively leptin may have a role in stimulating pro-TRH [260]. There is no evidence of benefit from levothyroxine treatment [325] and management should include lifestyle and dietary interventions to promote weight loss.

Reduced Thyroid Hormones Without TSH Elevation

Central Hypothyroidism

Congenital or acquired central hypothyroidism is usually associated with additional pituitary hormone deficits, although it can present in isolation. TSH is often detectable, although inappropriate for the low fT4 and fT3. It is important to establish full pituitary hormone status before treating with levothyroxine. In particular, cortisol deficiency must be treated for 48 hours before levothyroxine replacement. Levothyroxine increases metabolic rate and renal clearance and may precipitate an adrenal crisis if cortisol concentrations are inadequate.

Sick Euthyroid Syndrome

Sick euthyroid syndrome or non-thyroidal illness syndrome (NTIS) occurs during illness and patients present with low or low/normal fT4, low fT3 and raised rT3 without the expected rise in TSH. This may initially be protective with a reduction in metabolic requirements and energy conservation. fT3 may rise acutely due to reduced binding protein activity and changes in DIO1 and DIO3 activity [326] but subsequently fall with ongoing illness and fasting. There is downregulation of the hypothalamic–pituitary axis with alterations in TRH and TSH expression [327] and it is also thought that cytokines may directly inhibit thyroid hormone production and deiodinase activity [327]. Treatment with levothyroxine is unlikely to be of benefit.

Elevated Thyroid Hormones and Unsuppressed TSH

Causes of elevated thyroid hormones with unsuppressed TSH include assay interference due to qualitative changes in serum binding proteins (e.g. familial dysalbuminaemic hyperthyroxinaemia [FDH]), the presence of interfering

antibodies (heterophile, anti-TSH or anti-iodothyronine), clinical conditions such as non-thyroidal illness or psychiatric disorders and drugs (e.g. amiodarone, heparin and thyroxine replacement therapy) (Table 8.2). Such TFT patterns may also be seen in healthy neonates. Raised serum binding proteins typically result in changes in total but not free serum thyroid hormone concentrations but transient alterations in fT4 and fT3 have occasionally been observed [328]. A further differential includes a TSH-secreting pituitary adenoma, although this is rare in childhood [329].

The validity of elevated free hormone measurements can be confirmed in two-step or equilibrium dialysis assays; raised values in equilibrium dialysis assays usually exclude the presence of abnormal circulating binding proteins and anti-iodothyronine antibodies. Demonstration of preserved linearity when TSH is assayed in dilution argues against an artefactual TSH value. Other causes (non-thyroidal illness, thyroid hormone measurements made in the neonatal period, drugs) can usually be excluded by the clinical context.

Table 8.2 A summary of causes of elevated T4 and/or T3 with suppressed or unsuppressed TSH.

Disorder	fT4	fT3	TSH	Thyroid status	Additional clinical and biochemical features
Elevated thyroid hormones with suppressed TSH					
Graves' disease	⇑	⇑	⇓	Hyperthyroid	Eye disease, goitre, positive anti-TSH receptor antibody
Thyroiditis	⇑	⇑	⇓	Hyperthyroid	Goitre, raised inflammatory markers
Congenital hyperthyroidism (maternal transfer of antibodies, activating TSHR or Gsα mutation)	⇑	⇑	⇓	Hyperthyroid	Additional hormone excess, café au lait patches in McCune–Albright, goitre, negative anti-TSH receptor antibody
Excess levothyroxine ingestion	⇑	⇑	⇓	Hyperthyroid	
Elevated T4 and/or T3 with non-suppressed TSH					
Iodine deficiency[a]	⇔/⇓	⇑	⇔	Euthyroid	Goitre
Thyroid hormone biosynthetic defect[b]	⇔/⇓	⇔/⇑	⇔/⇑	Euthyroid	Goitre
FDH	⇑	⇔/⇑	⇔	Euthyroid	Normal equilibrium dialysis fT4
RTHbeta	⇑	⇑	⇔	Asymptomatic, variable hyperthyroid features	Goitre, tachycardia Growth retardation/failure to thrive, ADHD, normal SHBG level
RTHalpha	⇔/⇓	⇔/⇑	⇔	Variable tissue hypothyroidism	⇓/⇔ rT3, macrocephaly, growth retardation, motor dyspraxia, constipation, skin tags, characteristic facies
MCT8	⇔/⇓	⇑	⇔	Hepatic and muscle thyrotoxicity (muscle wasting, catabolism, ⇑SHBG)	⇓rT3, ⇑SHBG, mental and psychomotor retardation
SECISBP2	⇑	⇔/⇓	⇔	Euthyroid	⇑rT3, ⇓Se, growth retardation, hearing loss, infertility, muscular dystrophy, photosensitivity
TSHoma	⇑	⇑	⇔	Hyperthyroid	⇑SHBG, ⇑a-subunit/TSH molar ratio, flat TSH response to injected TRH, pituitary micro- or macroadenoma on MRI
Non-thyroidal illness[c]	⇑	⇑	⇔	Euthyroid	Intercurrent illness
Heparin	⇑	⇑	⇔	Euthyroid	Normal total T4
Amiodarone[d]	⇑	⇔	⇔/⇑	Euthyroid	
Antibody interference	⇔/⇑	⇔/⇑	⇔/⇑	Dependent on whether interference occurs in TSH or thyroid hormone assay	

[a] Iodine deficiency may be associated with overt hypothyroidism depending on severity.
[b] Thyroid hormone biosynthesis defects more commonly cause CH; however, T3/T4 ratio may be increased, as reported for TG mutations.
[c] Non-thyroidal illness more commonly causes decreased concentrations of thyroid hormones.
[d] Amiodarone may also cause overt hypo- or hyperthyroidism.

Drugs other than levothyroxine, which may be particularly relevant to pediatric practice, include heparin, which can result in artefactually raised fT4 and fT3 concentrations. This occurs due to heparin-induced activation of endothelial lipoprotein lipase *in vivo*, which then causes increased NEFA generation *in vitro* during sample storage or incubation. If NEFA concentrations exceed normal serum binding capacity, this results in direct competition for T4 and T3 binding sites on TBG and subsequent artefactual elevation of free hormone concentrations [330]. Furosemide, aspirin, NSAIDS and phenytoin may also mediate competition for TH binding sites on carrier proteins. Amiodarone may provoke overt thyrotoxicosis or hypothyroidism but it also impairs DIO1-mediated deiodination of T4 to T3 resulting in elevated T4 concentrations with normal T3 and normal or mildly elevated TSH [329].

After exclusion of artefactual or acquired causes, genetic aetiologies, including RTH action (e.g. RTHβ or RTHα), defects in thyroid hormone transport proteins (such as mutations in *MCT8*) or metabolism (*SBP2* mutations), should be considered. FDH, in which a mutation in the albumin (*ALB*) gene results in the generation of an abnormal form of the albumin molecule with increased affinity for T4 resulting in artefactually elevated free thyroid hormones by many assays, can be confirmed by molecular genetic studies.

Defective TH Action – Resistance to Thyroid Hormone (RTH)

RTHbeta

TRβ-mediated RTH in humans has an incidence of ~1 in 40,000 and arises due to point mutations in the *THRB* gene [331]. *THRB* gene mutations cluster in three different 'hotspots' in the LBD of the receptor with subsequent impairment of ligand binding or co-activator recruitment causing defective transcriptional function and dominant negative inhibition of wild-type receptor function [332–334]. Individuals with RTHβ exhibit a characteristic biochemical profile (raised fT4 and fT3 with unsuppressed TSH) due to refractoriness within the TRβ-dependent negative feedback signalling system that regulates the HPT axis. This results in the axis equilibrating around a new higher 'set point' with elevated fT4 and fT3 concentrations.

Clinical manifestations of RTHβ are highly variable and the majority of individuals are relatively asymptomatic. Associated clinical features may be attributable to differential TR isoform expression in different organs such that tissues predominantly expressing TRβ (liver, kidney, hypothalamus, pituitary) exhibit RTH, whereas tissues expressing predominantly TRα (skeletal and cardiac muscle, bone, brain) remain sensitive to the elevated circulating concentrations of thyroid hormones and so may exhibit relative thyrotoxicosis. Thus, RTH subjects have increased heart rate compared with euthyroid controls, which may reflect the predominance of TRα in myocardium, and increased resting energy expenditure probably mediated by the predominance of TRα in skeletal muscle. Conversely, serum SHBG levels (a hepatic marker of thyroid hormone action) are inappropriately normal or low and individuals may exhibit a mixed dyslipidaemia, reflecting hepatic RTH consonant with the fact that TRβ is the main receptor expressed in this tissue. Children with RTHβ may present with growth retardation or failure to thrive and ADHD may also be associated. Goitre is also common [66, 68, 335].

Individuals with heterozygous, TRβ-mediated RTH do not usually require treatment, as the thyroid hormone resistance is compensated by the elevated concentrations of thyroid hormones. In these individuals, a genetic diagnosis of RTHβ is nonetheless important to prevent inappropriate treatment recommendations, e.g. thyroid surgery or radioactive iodine due to incorrect diagnosis of GD. A minority of patients exhibit thyrotoxic symptoms, such as tremor or tachycardia, which may respond to beta blockade. In rare cases, treatment with triiodothyroacetic acid (Triac), a thyromimetic agent with a higher affinity for TRβ than TRα, may achieve lowering of TSH and serum thyroid hormone concentrations, thereby reducing clinical thyrotoxicity [66].

Rarely, homozygous *THRB* point mutations or deletions have been described in association with a more severe phenotype including marked intellectual impairment, hearing loss [336], dysmorphic features (birdlike facies, pigeon breast, winged scapulae), deaf–mutism and colour blindness [337].

RTHalpha

TRα-mediated RTH occurs as a result of heterozygous, dominant negative, loss-of-function mutations in the *THRA* gene [95]. It presents with tissue-specific hypothyroidism in tissues predominantly expressing the TRα isoform and near-normal TFTs since TRβ is the predominant isoform regulating TH negative feedback in the hypothalamus and pituitary gland. Patients may exhibit characteristic broad facies with hypertelorism, a flattened nose, prominent tongue and thick lips and are macrocephalic, perhaps due to delayed fontanelle closure. An excessive number of skin tags have been noted in many cases [95, 338–340].

Growth retardation is often prominent, in keeping with the crucial role of TRα for normal skeletal maturation and is commonly predominantly lower segmental. Radiological features of skeletal dysplasia in affected children may include Wormian bones (disordered, intramembranous ossification), epiphyseal dysgenesis

(disordered, endochondral ossification) and delayed dentition [95, 338–340]. Constipation is common and may be severe.

TRα is the predominant TR isoform in the brain and affected individuals usually exhibit neurocognitive deficits including delayed milestones (motor, speech) in childhood, impaired motor coordination and slow initiation of movement, manifesting as dyspraxia or broad-based gait and slow speech. IQ is variably reduced [341].

The commonest biochemical abnormalities include low/low–normal T4 and high/high–normal T3 concentrations, subnormal T4/T3 ratio and variably reduced reverse T3, which may reflect altered metabolism of thyroid hormones, for which two mechanisms have been hypothesized; first, in mice with a dominant negative TRα1 mutation (TRα1-PV), increased hepatic DIO1 levels augment T4 to T3 conversion; alternatively, reduced tissue levels of DIO3, whose expression is TRa1 regulated, may result in decreased inner ring deiodination of T4 to rT3 and T3 to T2. Additional biochemical abnormalities include mildly elevated muscle creatine kinase and mild, normocytic anaemia [341].

The initial *THRA* mutations involved the TRα1 (hormone binding) isoform alone. Subsequent descriptions of cases harbouring mutations affecting TRα1 and TRα2 report overlapping phenotypes with TRa1-specific mutations with no discernible additional features attributable to TRα2 loss of function [342, 343]. There is one report of a 27 year old female with a mutation affecting both TRα1 and TRα2 who exhibits additional skeletal features (micrognathia, clavicular agenesis, hypoplasia, metacarpal fusion and syndactyly) as well as hyperparathyroidism and chronic diarrhoea that have not been recorded in other RTHα cases; it remains unclear whether these are solely attributable to the TRa1 N359Y mutation. Additionally milder forms of RTHalpha have now been described, which may present with more subtle clinical features of hypothyroidism and high normal fT3, low normal fT4 and normal TSH. It has been suggested that RTHα phenotype severity may correlate to some extent with the location and degree of functional impairment incurred by the *THRA* mutation involved [344]. Although <30 RTHα cases have been reported to date, this syndrome may be more common, especially in milder forms, but as yet incompletely ascertained due to its modest biochemical abnormalities and its association with non-endocrine phenotypes [341].

Levothyroxine therapy has been beneficial in some childhood cases, improving growth, alleviating constipation and improving motor development and well-being [341, 343]. TSH concentrations suppress readily on treatment with elevation of fT3 to supraphysiological levels, which does raise the possibility that chronic excess TH exposure in thyroxine-treated RTHα patients might lead to unwanted toxicities in normal TRβ-containing tissues such as the liver or bone [95, 339].

Defective TH Metabolism – SECISBP2

The human selenoproteome comprises around 25 selenoproteins, which contain the rare amino acid selenocysteine (Sec) and include the family of deiodinase enzymes responsible for thyroid hormone metabolism. An intricate mechanism controls the incorporation of Sec into selenoproteins, because Sec is encoded by a UGA codon, which would usually function as a translation termination codon and must therefore be correctly recoded as the amino acid selenocysteine (Sec) instead of a premature stop. The interaction of the Sec insertion sequence (SECIS) binding protein 2 (SECISBP2) with a SECIS element in the 3′-untranslated region of selenoprotein mRNAs is instrumental in mediating this recoding process [66].

Biallelic mutations in SECISBP2 result in a characteristic biochemical signature comprising elevated T4, low/normal T3, high reverse T3 and normal/high TSH. This is thought to reflect impaired thyroid hormone metabolism due to deficiencies of the three key selenoprotein deiodinase enzymes [345–347]. However, since SECISBP2 in humans is required for synthesis of all selenoproteins, SECISBP2 deficiency results in a complex, multisystem phenotype. In the eight families reported to date, the most prominent feature is growth retardation. Sensorineural hearing impairment, myopathy (particularly axial) and impaired motor and intellectual development are variably reported.

Additional manifestations comprise eosinophilic colitis, rotary vertigo, male infertility due to impaired spermatogenesis, increased photosensitivity and increased body fat despite preserved insulin sensitivity or fasting ketotic hypoglycaemia [345, 346, 348–351].

Some phenotypes are probably related to tissue-specific selenoprotein deficiencies (e.g. SEPN deficiency causing myopathy) but others, such as cutaneous photosensitivity, hearing impairment and possibly insulin sensitivity, may be mediated by increased reactive oxidative species, reflecting the antioxidant function of many selenoproteins [345]. Additionally, selenoproteins with hitherto uncharacterized functions may play a role. Individuals also exhibit decreased serum selenium concentrations as a consequence of impaired synthesis

of selenoprotein P and glutathione peroxidase 3 (Gpx3), the major carriers of Se in serum. Treatment with triiodothyronine has been beneficial in some cases for improving growth [345, 349].

Although selenium supplementation raised serum selenium concentrations and, in some cases, SePP, it failed to normalize GPx activity or serum TH abnormalities, arguing against long-term benefits [352]. Given the putative role of increased reactive oxidative species in this syndrome, studies of antioxidant therapy are warranted. Recently, a homozygous mutation in the transfer RNA, tRNA[Ser]Sec, required for selenocysteine incorporation was identified in a child with a similar thyroid biochemical phenotype and muscle weakness, with associated impaired selenoprotein biosynthesis, which seemed selectively to involve stress-related rather than housekeeping selenoproteins [353].

Defective Thyroid Hormone Transport – MCT8

Inactivating mutations of the thyroid hormone transport protein MCT8 (*SLC16A2)* result in the rare X-linked psychomotor retardation syndrome known as Allan–Herndon–Dudley syndrome (AHDS) [354]. Patients with this disease have global developmental delay and profound intellectual disability, usually with an IQ <40; most are unable to speak. Severe neuromotor impairment manifests as central hypotonia, with spastic paraplegia developing with advancing age; most patients are unable to sit or stand independently and all have swallowing difficulties. MRI imaging shows transient delayed myelination or dysmyelination [65]. Weight usually decreases during childhood and muscle mass declines progressively [65, 66].

Thyroid biochemistry associated with MCT8 mutations comprises markedly elevated T3 concentrations, low or low–normal T4 and decreased rT3 concentrations, with normal or slightly raised concentrations of TSH. Data from murine models suggests that the pathophysiology underlying this hormonal signature may be complex, including increased renal trapping of T4, increased consumption of T4 due to elevated renal and hepatic DIO1 expression and impaired thyroid hormone secretion from the thyroid gland [36, 355–357].

The clinical manifestations of MCT8 deficiency remain incompletely understood; peripheral thyrotoxicity arising from elevated circulating concentrations of T3 may result in increased catabolism, weight loss and muscle wasting, since T3 can enter the cells of some peripheral tissues via transporters other than MCT8. Hepatic SHBG production is also increased. In the brain, hypothyroidism resulting from impaired T3 entry into MCT8-expressing neuronal cells may impair myelination and neuronal differentiation [65, 66]. Although female carriers of MCT8 mutations do not exhibit a neurological phenotype in the absence of skewed X chromosome inactivation, their fT4 concentrations are generally between those of unaffected individuals and affected males [66].

Unfortunately, there is no effective current treatment for AHDS. Management of the neurological phenotype is supportive, with no evidence that thyroxine treatment offers any benefit [358, 359], although combination treatment with PTU and L-T4 may improve body weight and enable a reduction in SHBG. There is interest in the role of thyromimetics in ameliorating the peripheral thyrotoxicity associated with MCT8 mutations [65, 66].

Familial Dysalbuminaemic Hyperthyroxinaemia (FDH)

Albumin has multiple ligand-binding sites including a T4-binding site in subunit IIA, for which the side chains of flanking amino acids require minor rearrangements in order to accommodate T4. These conformational changes predominantly involve W214, R222 and R218, which project from the helix that forms one side of the mouth of the pocket and make direct van der Waal contacts with T4 [360].

Specific genetic mutations in these amino acids (p.R218H, p.R218P, p.R218S, p.R222I) are associated with increased binding affinity for thyroxine due to decreased steric hindrance resulting in euthyroid hyperthyroxinaemia inherited in an autosomal dominant fashion [361]. p.R218H, p.R218S and p.R222I are associated with predominantly raised TT4 concentrations. p.R218P results in markedly elevated TT4 and raised TT3 concentrations and an additional mutation, p.L66P, is associated with predominant hypertriiodothyroninaemia [362–364].

Individuals with FDH are clinically euthyroid, since the mutant albumin results in the establishment of a new equilibrium between bound and free hormone, such that free hormone concentrations remain normal in the context of increased bound hormone. However, there is potential for misdiagnosis of thyrotoxicosis in this setting, as total thyroid hormone measurements are elevated and standard laboratory methods for the measurement of free thyroid hormones may give falsely elevated results due to assay interference by the mutant albumin. Such misdiagnosis may result in the recommendation of inappropriate surgical or medical therapy [365, 366].

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9

The Adrenal Cortex and Its Disorders

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KEY LEARNING POINTS

- Steroid synthesis is the key function of the adrenal cortex. Three main groups of hormones are produced: mineralocorticoids from the zona glomerulosa, glucocorticoids from the zona fasciculata and androgens from the zona reticularis. Unlike many other hormones, steroids are not stored in the adrenal gland but are synthesized and released in response to appropriate stimuli.
- Mineralocorticoids, principally aldosterone, regulate renal sodium retention influencing electrolyte balance, intravascular volume and blood pressure. The main factor controlling aldosterone is angiotensin through the renin–angiotensin–aldosterone system.
- Glucocorticoids, principally cortisol, are named for their carbohydrate mobilizing activity, but exert widespread effects throughout the body; at least 10% of all genes are influenced by glucocorticoid concentrations. Cortisol secretion is an example of a classical endocrine feedback loop as it is under negative feedback control from the dynamic and tightly regulated hypothalamic–pituitary–adrenal axis.
- Adrenal androgens modulate the mid-childhood growth spurt and regulate some sexual characteristics in women such as androgen-dependent hair growth.
- Advances in molecular genetics have helped to elucidate the pathological basis of many adrenal disorders and more than 40 distinct single-gene disorders that affect adrenal function have been identified.
- Congenital adrenal hyperplasia (CAH) secondary to 21-hydroxylase deficiency is the most common cause of adrenal insufficiency in children. Rare conditions do occur and reaching a specific diagnosis can have important implications in terms of treatment, long-term outcome, associated features and recurrence risk.
- CAH is an umbrella term for defects in several enzymes involved in the steroidogenic pathway. The precise mode of presentation and clinical and biochemical features are determined by the nature and severity of the block.
- 21-Hydroxylase deficiency can be associated with salt-losing, simple virilizing and non-classical CAH.
- CAH needs careful lifelong clinical and biochemical monitoring. Current treatment regimens do not permit physiological replacement and therefore treatment approaches aim to control symptoms of adrenal insufficiency and minimize any risk of adrenal crisis while also reducing any side effects of overtreatment.
- Adrenoleukodystrophy should be considered in any boy who presents with adrenal insufficiency.
- Excessive exposure to glucocorticoids causes Cushing syndrome and can lead to adrenal suppression after 10 days; steroid withdrawal requires careful attention to detail.
- The most common cause of Cushing syndrome is iatrogenic steroid administration followed by Cushing disease secondary to a pituitary microadenoma, but in preschool children adrenal tumours predominate.
- The diagnosis and management of Cushing syndrome can be extremely difficult in children and adolescents but the two earliest, most reliable features are growth failure and weight gain.

Introduction

Adrenal disorders can be difficult to diagnose and are associated with considerable morbidity and mortality if undiagnosed.

The adrenal cortex produces three groups of steroid hormones: mineralocorticoids (e.g. aldosterone) regulate salt retention and blood pressure, glucocorticoids (e.g. cortisol) contribute to many metabolic processes and are needed for the maintenance of blood glucose and well-being, and adrenal androgens (e.g. dehydroepiandrosterone [DHEA]) influence androgen-dependent hair growth (Figure 9.1). The adrenal medulla produces adrenaline and noradrenaline needed for an acute stress response.

Brook's Clinical Pediatric Endocrinology, Seventh Edition. Edited by Mehul T. Dattani and Charles G. D. Brook.

Companion website: www.wiley.com/go/dattani/brookcpe7

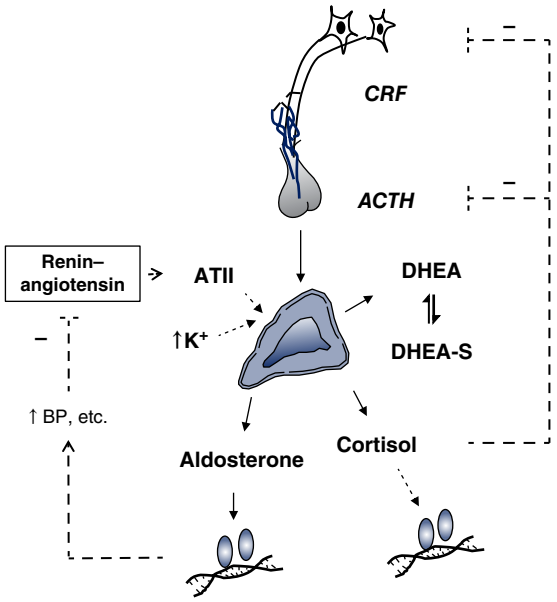


Figure 9.1 Simplified overview of the hypothalamo–pituitary–adrenal axis showing the key steroid hormones and feedback mechanisms. BP – Blood Pressure. *Source:* Modified from Achermann [1] © 2004 Blackwell Publishing Limited.

Adrenocortical disorders result from insufficiency of one or more of these adrenal steroids or from adrenal steroid excess. The clinical and biochemical features at presentation and the natural history of the condition vary depending on which adrenal steroids are affected, as well as the underlying pathological condition.

In this chapter we provide an overview of the development, function and regulation of the adrenal gland, the causes, diagnosis and management of adrenal insufficiency (AI) and of conditions causing adrenal hormone excess, and information for families and young people with adrenal conditions.

Development, Function and Regulation of the Adrenal Gland

History of Adrenal Medicine

The adrenal glands were long overlooked by anatomists and were described for the first time by the Italian anatomist Bartolomeo Eustachius in 1563. Their biological role became apparent when Addison described the effects of AI in the middle of the 19th century and animal studies by Brown–Séquard reported the consequences of adrenalectomy.

Harvey Cushing was first to report the classic features of steroid hormone excess in 1932. During that decade it became clear that the adrenal cortex produced two broad categories of steroids termed 'glucocorticoids' and 'mineralocorticoids'. Soon after, the laboratories of Reichstein and Kendall isolated and characterized the structure of several key adrenal steroids, for which they jointly received the Nobel Prize in Medicine in 1950.

Advances in molecular genetics have helped elucidate the pathological basis of many adrenal conditions and more than 40 distinct single gene disorders have been shown to affect adrenal function. Nevertheless, all clinical adrenal disorders need to be viewed within the context of the development and physiological function of the hypothalamic–pituitary–adrenal (HPA) axis, one of most dynamic and tightly regulated endocrine systems. Therefore, an understanding of the development and function of this axis is necessary.

Development and Anatomy of the Adrenal Gland

Adrenal Development

Adrenal development starts in the very early weeks of human embryonic life but little is known about this complex process. The adrenal cortex develops from a condensation of intermediate mesoderm at around 4 weeks' post-conception (wpc). This tissue is known as the adrenogonadal primordium as it gives rise to the steroidogenic cells of the gonad as well as those of the adrenal gland (Figure 9.2). At around 5 wpc, the bipotential gonad separates from this region and migrates caudally to develop into either a testis or ovary, while the primitive adrenal gland migrates retroperitoneally and becomes associated with the upper pole of the kidney. Sympathetic neural cells derived from the neuroectoderm migrate into the developing adrenal gland at around 7 wpc. Initially these cells are scattered in the developing structure but coalesce eventually to form the adrenal medulla. The adrenal capsule forms at around 8 wpc [2].

The adrenal gland is initially heterogeneous but the cortex organizes into an outer 'definitive zone' (DZ) and a much larger inner 'fetal zone' (FZ) during the early stages of development. An additional transition zone may be seen from around 14 wpc. The DZ contains cells potentially able to synthesize glucocorticoids and mineralocorticoids but have relatively low expression of 3β-hydroxysteroid dehydrogenase type II (3β-HSD2) (*HSD3B2*) (see 'Steroidogenesis'). Upregulation of *HSD3B2* around 8–9 wpc is thought to result in transient cortisol synthesis and a functionally intact HPA axis but this process diminishes in the second trimester. The large cells of the FZ have high expression of 17α-hydroxylase (P450c17) and relatively little 3β-HSD2, which results in the production of very large amounts of DHEA (and DHEA sulphate [DHEA-S]). This steroid is the hallmark of the fetal adrenal zone and can get converted to oestrogens by placental aromatase. Fetal adrenal steroids fall

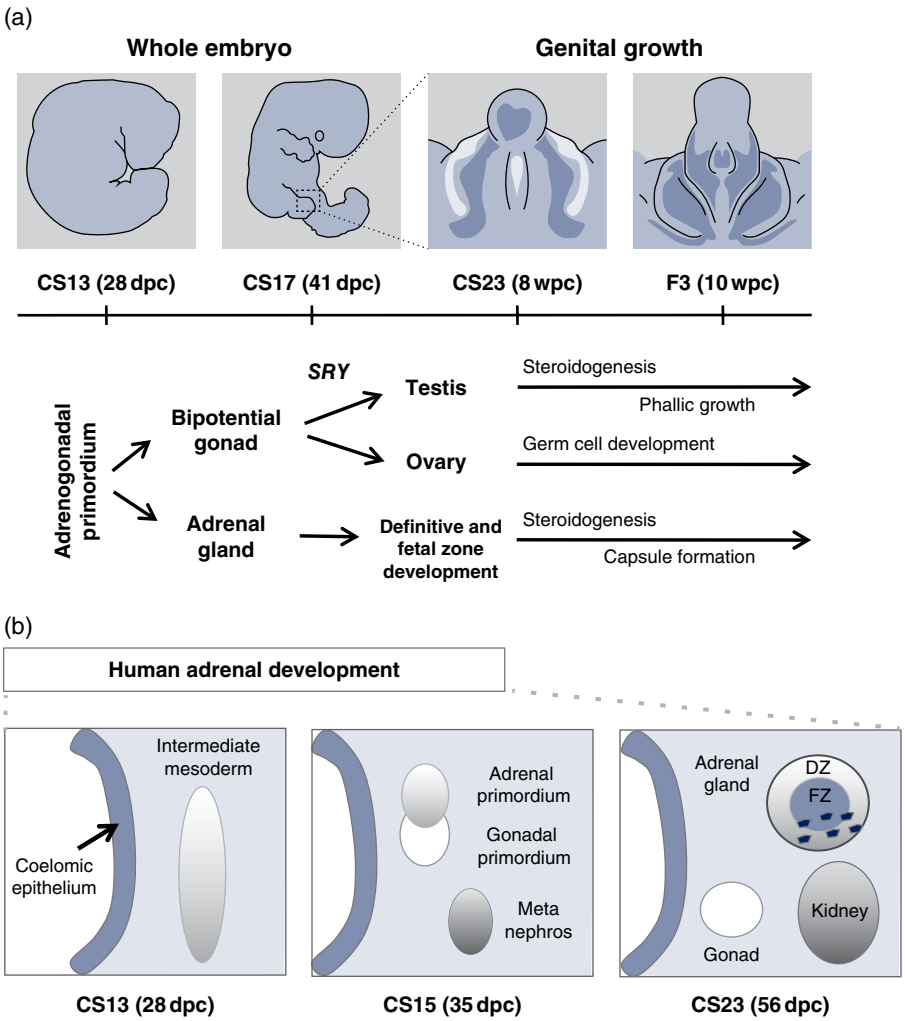


Figure 9.2 Overview of human adrenal development. (a) Key events in adrenal and gonad development in embryonic and early fetal life. dpc, days post-conception; wpc, weeks post-conception. (*Source:* Reproduced from del Valle et al. [2]. Licensed under CC BY SA v4, https://creativecommons.org/licenses/by/4.0) (b) Cartoon showing the anatomical relation of the adrenal gland, gonad and kidney during embryonic development. DZ, definitive zone; FZ, fetal zone. Chromaffin cells are shown in dark grey.

after birth and the FZ regresses by 6–12 months of age [3]. The teleological significance of the fetal adrenal zone, which is found only in higher primates, is unknown.

The adrenal glands in the human fetus are massive compared with their size in adults, primarily due to the extensive FZ. From the early stages of development the adrenal glands are as large as the developing kidney and are roughly the same size as the adult adrenal glands at birth (combined weight 8–9 g or 0.5% of total body weight) (Figure 9.3). *Relative* adrenal weight falls postnatally due to general somatic growth so that the adrenal glands comprise only 0.02% of adult body weight.

Knowledge about the factors that control adrenal development or the way in which adrenal stem cells contribute to adrenal growth is limited. An array of transcription factors and signalling molecules is necessary for adrenal development as shown in humans with adrenal hypoplasia and in mouse models of adrenal agenesis and studies of gene expression during early human adrenal development [4].

Two key nuclear receptors involved are steroidogenic factor 1 (SF-1) (officially known as NR5A1) and DAX-1 (officially known as NR0B1). Deletion of the gene encoding Sf-1 in the mouse results in complete adrenal and gonadal dysgenesis and severe disruption of SF-1 in humans can cause adrenal dysfunction, although most pathogenic variants in SF-1 in humans cause impaired testicular development and Leydig cell dysfunction rather than AI.

Pathogenic variants or deletions of DAX-1 in humans are well established as the cause of X-linked adrenal hypoplasia congenita (AHC) [5, 6]. Other factors causing adrenal hypoplasia in humans include cell cycle and growth regulators (CDKN1C, SAMD9) or signalling

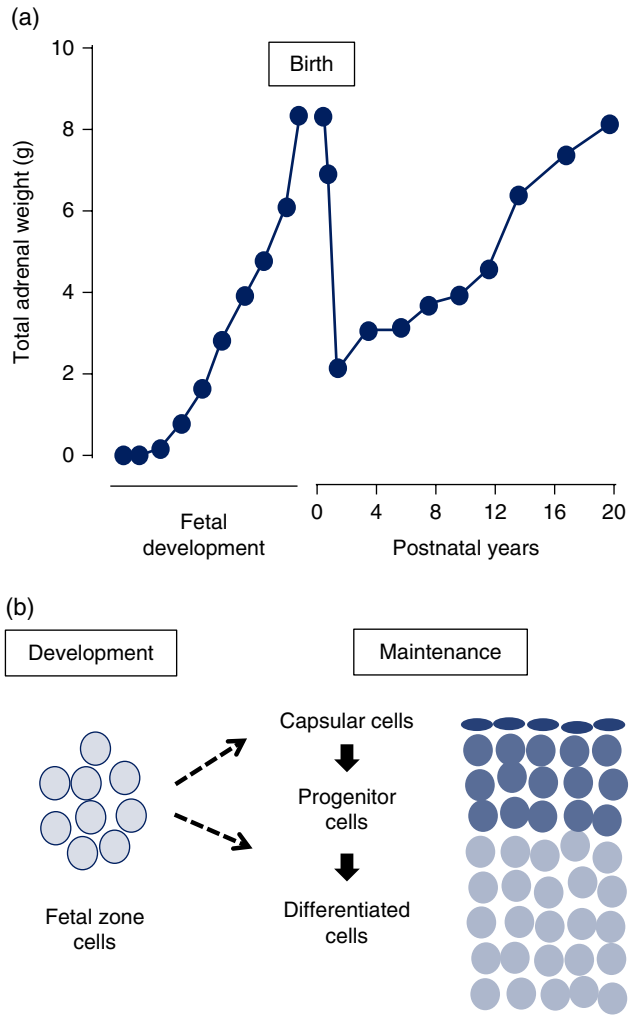


Figure 9.3 (a) Typical total combined adrenal weight in humans from early development through to young adulthood. The adrenal glands grow rapidly prenatally due to expansion of the large fetal zone, which involutes in the first few months after birth. (b) Cartoon showing proposed mechanisms of adrenal cell differentiation during development and maintenance.

molecules (WNT4) but other transcription regulators have been implicated in adrenal development in mouse models (Pbx1, Cited2, Tcf21, Wt1). Trophic factors such as insulin-like growth factor 2, ACTH and downstream ACTH signalling pathways are also important for the general growth of the adrenal gland.

Regulation of adrenal stem cell growth and differentiation is also poorly understood but has important implications for understanding adrenal tumours as well as developmental disorders. Some researchers propose that there is a common pool of subcapsular progenitor stem cells that differentiate through the different cell lineages as they move centripetally through the gland. This theory is supported by lineage tracing experiments in mice, which show that all mature cortical cells are derived from cells in the outer *zona glomerulosa*. Others propose that there is a population of stem cells in the FZ or transitional zone that can migrate in both directions to supply fetal and definitive cells. A combination of these theories may be correct, with the FZ contributing to subcapsular progenitor cells during development that then differentiate through the various zones to maintain stable cell populations in the mature gland (Figure 9.3). Some conditions associated with adrenal hypoplasia (e.g. IMAGe syndrome, X-linked AHC) may reflect a defect in progenitor cell expansion during early fetal development.

Anatomy and Structure of the Adrenal Gland

The mature adrenal glands are located in perinephric fat on top of the kidneys in a retroperitoneal position at the back of the abdomen. They are divided into an outer cortex and inner medulla surrounded by a capsule (Figure 9.4).

The cortex comprises three histologically distinct zones (Figure 9.4). The outer *zona glomerulosa* predominantly produces mineralocorticoids; the middle *zona fasciculata* mostly synthesizes glucocorticoids such as cortisol; the inner *zona reticularis* is involved in adrenal androgen synthesis.

Although these three zones generally have anatomically and functionally distinct roles, there is overlap in the regions as some groups of cells interdigitate and steroid output may vary and the contribution of different zones

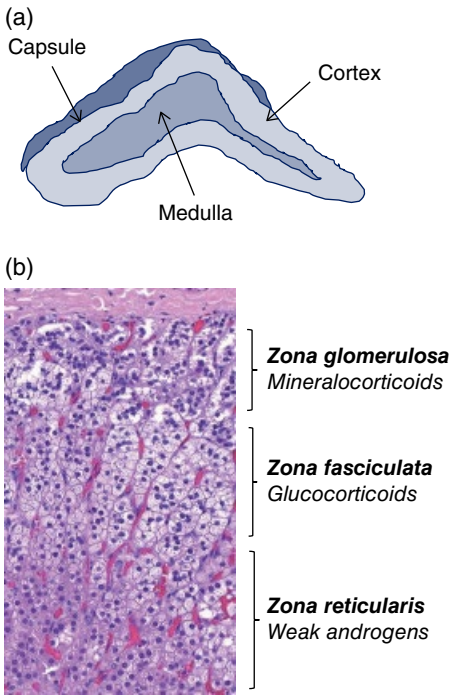


Figure 9.4 (a) Cartoon showing the structure of the adult adrenal gland. (b) Histology showing the three main zones of the mature adult adrenal gland (H&E stain, 40× magnification).

changes with age. The *zona glomerulosa* and *zona fasciculata* become differentiated in early childhood, whereas the *zona reticularis* develops in later childhood around the time of adrenarche and may develop fully only after puberty. In early adulthood the three zones account for ~15% (glomerulosa), 75% (fasciculata) and 10% (reticularis) of adrenal cortical volume.

The adrenal medulla initially forms at the periphery of the gland during development but fills the central region of the gland as it matures (Figure 9.4). The medulla contains sympathetic neuronal chromaffin cells that release adrenaline (epinephrine) and noradrenaline (norepinephrine). The adrenal medullary hormones allow a very rapid initial response to stress.

The blood flow to the adrenal glands is unusual as the arteries and veins do not run in parallel. Arterial blood flow to the adrenal glands comes through many small arteries from the phrenic and renal arteries, as well as from the aorta and even the ovarian and left spermatic arteries. Arterial blood enters the sinusoids in the adrenal cortex and flows towards the medulla, which is important because cortisol facilitates adrenaline synthesis. Venous drainage from the adrenal gland is from the right adrenal vein into the vena cava and left adrenal vein into the left renal vein. The adrenal gland has one of the greatest blood flows per gram of tissue of all the organs in the body.

Steroidogenesis

Structure of Steroids and Nomenclature

All steroid hormones are derivatives of pregnenolone, which is derived from cholesterol and is the first precursor in the steroidogenic pathway (Figure 9.5).

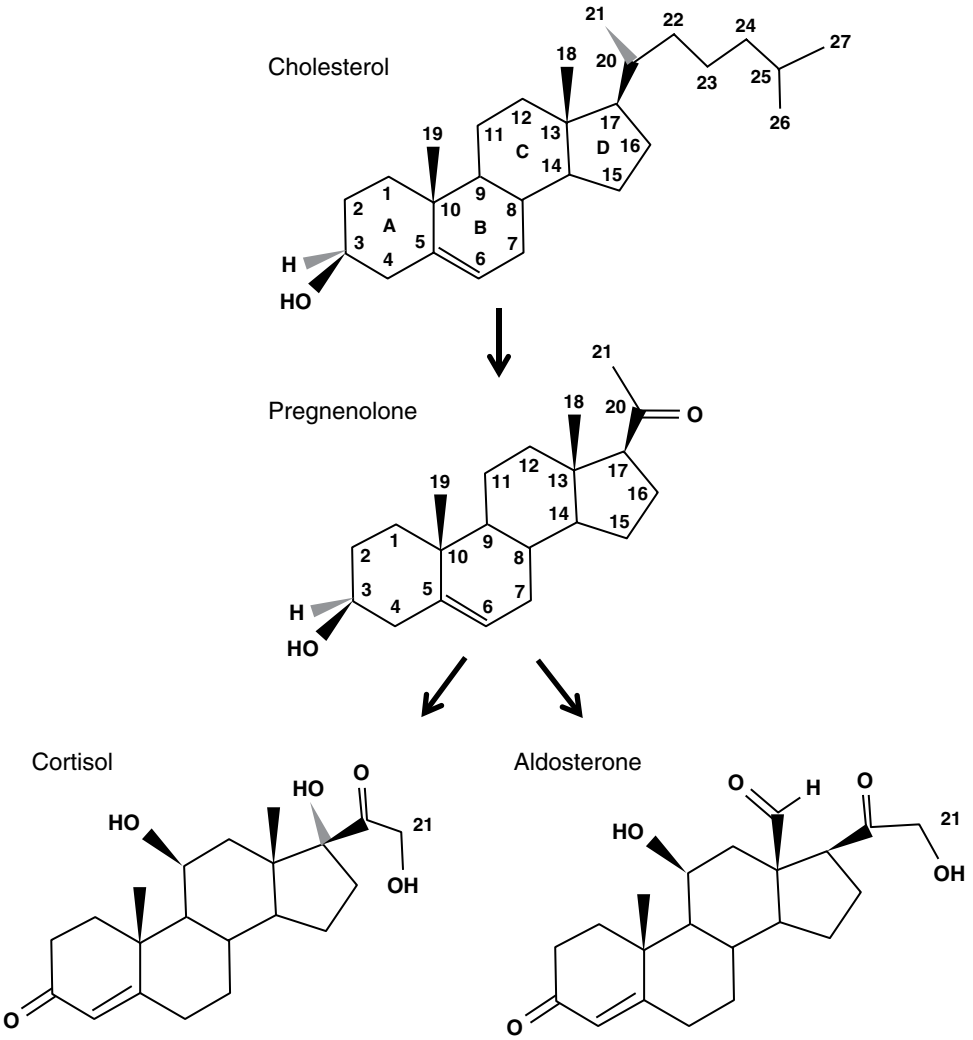


Figure 9.5 The chemical structures of cholesterol and three other important adrenal steroids (pregnenolone, cortisol and aldosterone). Key carbon atoms are labelled 1–21.

Pregnenolone and its derivatives are termed C_{21} steroids as they contain 21 carbon atoms. Each carbon atom is numbered, indicating the location at which the various steroidogenic reactions occur (e.g. 21-hydroxylation, 11-hydroxylation).

C_{19} steroids are generated by the 17,20-lyase activity of P450c17 that cleaves the bond between carbon atoms 17 and 20. C_{19} steroids include all the androgens, such as DHEA, androstenedione and testosterone. P450 aromatase converts C_{19} androgens to C_{18} oestrogens.

Except for oestrogens, all steroid hormones have a single unsaturated carbon–carbon double bond. Steroids having this double bond between carbon atoms 4 and 5, including all the principal biologically active steroids, are termed Δ^4 steroids; their precursors, having a double bond between carbon atoms 5 and 6, are termed Δ^5 steroids (Figure 9.5). The two isozymes of 3β-hydroxysteroid dehydrogenase (3β-HSD) convert Δ^5 to Δ^4 steroids.

Official steroid nomenclature describes the chemical name of the compounds and is lengthy (e.g. cortisol is 11β,17α,21-trihydroxypregn-4-ene-3,20-dione). Therefore, we use only the standard trivial names in this chapter. Older nomenclature describes the migration of steroids during chromatography (e.g. cortisol is 'compound F'). This approach is now outdated.

The Biochemistry of Steroidogenesis

Steroid synthesis is the key function of the adrenal gland. Unlike many other hormones, such as peptide hormones, steroids are not stored and must be synthesized and released in response to stimulation by ACTH (predominantly glucocorticoids/androgens) and angiotensin II (predominantly mineralocorticoids) (Figure 9.1).

In an acute situation, ACTH stimulates rapid *de novo* synthesis and release of steroids within minutes. In more chronic situations, such as in times of ongoing stress, ACTH plays a role in upregulating the transcription of steroidogenic enzyme genes and steroidogenic machinery.

Steroid synthesis begins with uptake (or mobilization) of cholesterol and ends with release of the appropriate steroid into the circulation. A simplified overview of steroidogenesis in the adrenal gland is shown in Figure 9.6.

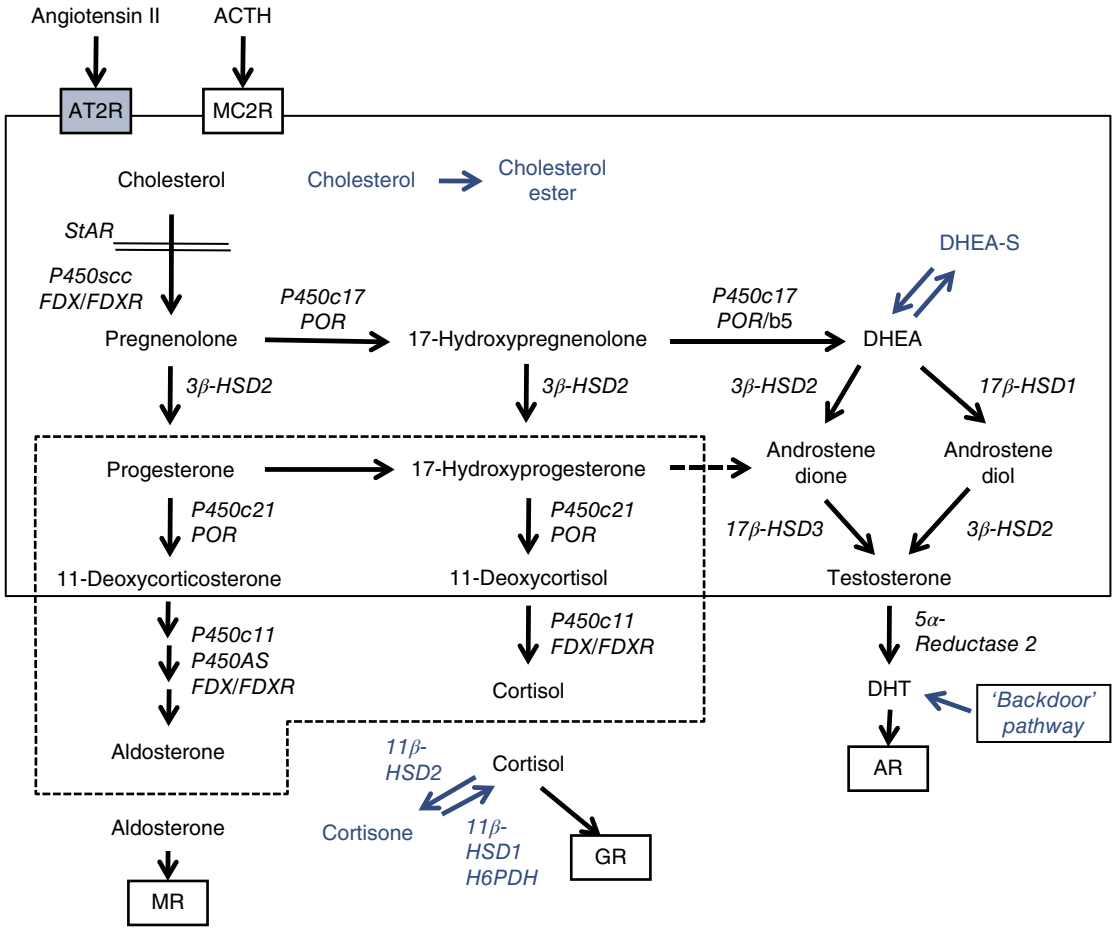


Figure 9.6 Simplified overview of steroidogenesis in the adrenal gland and targets of steroid hormone action. MR, mineralocorticoid receptor; GR, glucocorticoid receptor; AR, androgen receptor.

Here, we will describe the mechanisms involved in cholesterol uptake and transport into mitochondria, the different families of steroidogenic enzymes and the role each enzyme plays in steroid synthesis [7]. Several enzymes that are not expressed in the adrenal gland are also important in mediating the peripheral effects of steroidogenesis and steroid hormone action and these will be described briefly too. Finally, it is important to remember that many of the core components of steroidogenesis are also involved in sex steroid synthesis in the testis and ovary. Thus, the phenotypes that result from disruption of many of these enzymes can include gonadal as well as adrenal features.

The Major Groups of Steroidogenic Enzymes: Cytochrome P450s and Hydroxysteroid Dehydrogenases

The two major groups of steroidogenic enzymes are *cytochrome P450s* and *hydroxysteroid dehydrogenases* (HSDs) (Table 9.1). Most key steroidogenic enzymes belong to the cytochrome P450 family of oxidative enzymes that are ~500 amino acids in length and contain a single haem group. They derived their name (P450 or pigment 450) from their absorption of light at 450 nm in the reduced state. The cytochrome P450 family contains 57 enzymes in humans. They play a wide-ranging role in metabolism (e.g. vitamin D metabolism) as well as in the degradation of many endogenous and exogenous drugs, toxins and environmental chemicals, usually in the liver. Five specific P450 enzymes are involved in adrenal steroidogenesis: P450scc, P450c17, P450c21 and P450c11 (with the isoforms P450c11β and P450c11AS). P450aro is expressed in the gonads and other tissues, where it catalyses the conversion of androgens to oestrogens.

HSDs are the other main class of enzyme involved in steroidogenesis. These do not contain a haem group but need NAD^+ or $NADP^+$ as cofactors. Most HSDs have several isoforms that catalyse reactions in different tissues or that catalyse the forward or reverse reactions of a specific chemical change. The main HSDs involved in adrenal steroid synthesis or peripheral modification of steroids are the 3β-HSD and the two 11β-hydroxysteroid dehydrogenases (11β-HSD), whereas the17β-hydroxysteroid dehydrogenases (17β-HSDs) regulate androgen synthesis (especially in the gonad) as well as other reactions. The HSD enzymes can be subdivided into the short chain dehydrogenase/reductase (SDR) family and the aldo/keto reductase (AKR) family.

Cholesterol Uptake, Storage and Mitochondrial Transport

Most cholesterol needed for steroid synthesis comes from plasma low-density lipoproteins (LDL) derived from dietary cholesterol. These LDL cholesterol esters

Table 9.1 Key factors and enzymes involved in adrenal steroidogenesis.

Protein	Activity	Gene	Partner	Localization
Steroidogenic acute regulatory protein	Facilitates cholesterol transport	*STAR*	—	Mitochondrion (outer membrane)
P450scc	Cholesterol side-chain cleavage	*CYP11A1*	FDX/FDXR	Mitochondrion (inner membrane)
3β-HSD2	3β-Hydroxysteroid dehydrogenase type II	*HSD3B2*	—	Endoplasmic reticulum
P450c17	17α-Hydroxylase	*CYP17A1*	POR	Endoplasmic reticulum
P450c17	17,20-Lyase	*CYP17A1*	POR, cytochrome b5	Endoplasmic reticulum
P450c21	21-Hydroxylase	*CYP21A2*	POR	Endoplasmic reticulum
P450c11β	11β-Hydroxylase	*CYP11B1*	FDX/FDXR	Mitochondrion (inner membrane)
P450c11AS	Aldosterone synthase	*CYP11B2*	FDX/FDXR	Mitochondrion (inner membrane)
17β-HSD3 (and 1)	17β-Hydroxysteroid dehydrogenase type III (type I)	*HSD17B3 (1)*	—	Endoplasmic reticulum
SULT2A1	Sulphotransferase	*SULT2A1*	—	Cytosol
PAPSS2	3′-Phosphoadenosine-5′-phosphosulphate synthase 2	*PAPSS2*	—	Cytosol
11β-HSD1	11β-Hydroxysteroid dehydrogenase type I	*HSD11B1*	H6PDH	Endoplasmic reticulum
11β-HSD2	11β-Hydroxysteroid dehydrogenase type II	*HSD11B2*	—	Endoplasmic reticulum

are taken up by the LDL receptor following receptor-mediated endocytosis and are then stored or converted to free cholesterol and used for steroid hormone synthesis. Storage of cholesterol esters in lipid droplets is controlled by the actions of two opposing enzymes: hormone-sensitive lipase (HSL) (previous known as cholesteryl ester hydrolase) and sterol O-acyltransferase (SOAT1) (also known as acyl coenzyme A [CoA], cholesterol acyltransferase [ACAT] or cholesterol ester synthetase). In addition, the adrenal gland can synthesize cholesterol *de novo* from acetate by 3-hydroxy 3-methylglutaryl coenzyme A (HMG-CoA) reductase but this is a relatively minor pathway for cholesterol production. Overall cholesterol biosynthesis is regulated by the sterol response element binding proteins (SREBPs).

ACTH stimulation results in the uptake of LDL cholesterol, increases free cholesterol (by stimulating HSL and inhibiting SOAT1) and can stimulate HMG-CoA activity. All these mechanisms increase availability of free cholesterol available for steroid synthesis.

The acute onset of steroidogenesis in response to stimulation of adrenal cells by ACTH (cortisol) or angiotensin II (aldosterone) requires rapid transport of cholesterol from the cytoplasm into the mitochondria. The main protein that mediates this transport is known as steroidogenic acute regulatory protein (StAR). StAR is located on the outer mitochondrial membrane with its amino-terminal (N-terminal) tail buried in the lipid bilayer. StAR mediates the movement of cholesterol from the outer to the inner membrane, thereby allowing access to the first enzyme in steroidogenesis, P450scc. Complete disruption of StAR in humans is associated with congenital lipoid adrenal hyperplasia (CLAH) (severe salt-losing AI and absent sex steroid production), whereas partial defects in StAR cause a predominant glucocorticoid effect (cortisol insufficiency).

In addition to StAR-mediated steroidogenesis, ~15% of cholesterol transport can result from StAR-independent events. This movement may occur by spontaneous cholesterol flux or through other potential mediators of cholesterol transport (e.g. the peripheral benzodiazepine receptor). The exact mechanisms involved in this process are poorly understood.

P450 Cholesterol 'Side-Chain Cleavage' (P450scc, CYP11A1)

The first step in steroid synthesis is mitochondrial conversion of cholesterol to pregnenolone (Figure 9.6), which is also the rate-limiting reaction. This conversion involves 20α-hydroxylation, 22-hydroxylation and cleavage of the cholesterol side chain to generate pregnenolone and isocaproic acid (Figure 9.5). Originally it was felt that three separate enzymes were responsible for these events but it was later shown that P450scc (named after 'side-chain cleavage' of cholesterol) catalysed all three reactions. P450scc is encoded by the gene *CYP11A1* located on chromosome 15 in humans. P450 mediates all three reactions through a single active site. Complete deletions of *Cyp11a1* in mice or severely disruptive mutations in P450scc in humans cause salt-losing AI and impaired sex steroid synthesis. Partial defects in P450scc cause predominantly glucocorticoid insufficiency like partial defects in StAR.

Electron Transport by Adrenodoxin Reductase and Adrenodoxin

P450scc oxidase activity requires electron transport that involves two key proteins, adrenodoxin reductase and adrenodoxin. Adrenodoxin reductase is a flavoprotein bound to the inner mitochondrial membrane that accepts electrons from NADPH (the reduced form of nicotinamide adenine dinucleotide phosphate) (Figure 9.7). Adrenodoxin reductase then transfers the electrons to adrenodoxin, which is found in the mitochondrial matrix or loosely bound to the inner mitochondrial membrane, by forming a 1:1 complex. Adrenodoxin transfers the electrons to P450scc through a similar 1:1 complex. This electron shuttle system is a generic electron transport system for all mitochondrial

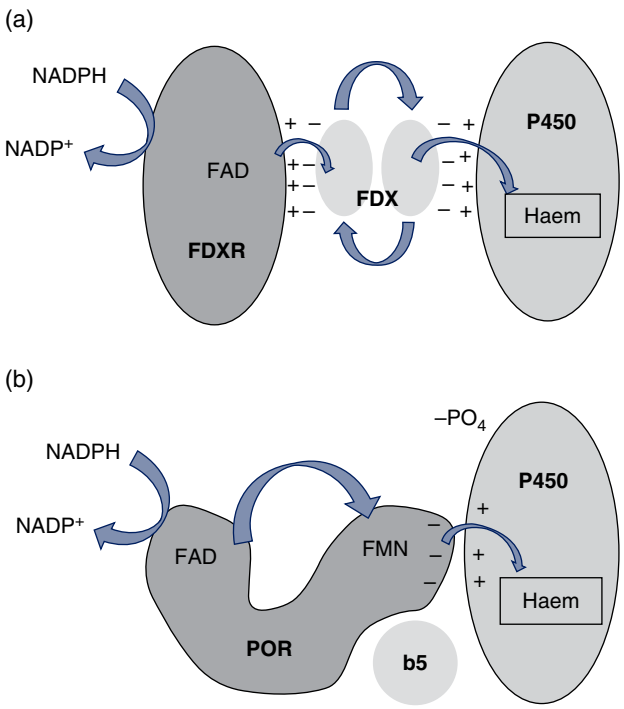


Figure 9.7 The two electron transfer systems for haem-containing cytochrome P450 enzymes. (a) Electron transfer via ferredoxin reductase (FDXR) (also known as adrenodoxin reductase) and ferredoxin (FDX) (adrenodoxin). (b) Electron transfer via P450 oxidoreductase (POR) (shown here with cytochrome b5).

P450 enzymes, including P450c11 in the adrenal and many P450 enzyme systems in other cells. Therefore, the official names of these proteins are ferredoxin reductase (FDXR) and ferredoxin (FDX).

3β-Hydroxysteroid Dehydrogenase

Once pregnenolone is produced from cholesterol, it can undergo 17α-hydroxylation by P450c17 to generate 17-hydroxypregnenolone (see below) or it may be converted to progesterone by 3β-HSD (Figure 9.6). 3β-HSD is a microsomal enzyme present in the endoplasmic reticulum that catalyses both the conversion of the hydroxyl group on carbon 3 to a keto group and the isomerization of the double bond from the B ring (Δ^5 steroids) to the A ring (Δ^4 steroids) (Figure 9.5). 3β-HSD2 is the isoform of the enzyme expressed in the adrenal gland and gonads. This enzyme converts pregnenolone to progesterone, 17α-hydroxypregnenolone to 17α-hydroxyprogesterone, DHEA to androstenedione and androstenediol to testosterone (Figure 9.6). The other isoform of the enzyme, 3β-HSD type I, is expressed in the placenta, breast and other tissues. Defects in the *HSD3B2* gene result in 3β-HSD2 deficiency. This condition causes glucocorticoid insufficiency with or without salt loss. Reduced androgen production is associated with hypospadias or reduced androgenization in 46,XY children, whereas the mild androgenic effects of excess adrenal androgens can cause clitoral enlargement and mild virilization in 46,XX children.

P450c17 (17α-Hydroxylase)

P450c17 is an enzyme bound to the smooth endoplasmic reticulum that has both 17α-hydroxylase and C-17,20-lyase activities. This reaction requires electron transport from a related protein, P450 oxidoreductase (POR). The 17α-hydroxylation reaction can convert pregnenolone to 17α-hydroxypregnenolone as well as progesterone to 17α-hydroxyprogesterone (17-OHP) (Figure 9.6). The C-17,20-lyase reaction involves cleavage of the #c17,20 carbon bond that converts 17α-hydroxypregnenolone to DHEA and requires the cofactor cytochrome b5 (CYB5). This reaction can also convert 17-OHP to androstenedione but this is a minor pathway in humans with only 3% the rate of conversion of 17α-hydroxypregnenolone to DHEA.

P450c17 is a key *branch point* enzyme because of its position in the steroid pathway and because of its dual function. Cells of the *zona glomerulosa* lack P450c17, resulting in the conversion of pregnenolone to aldosterone. Cells of the *zona fasciculata* have 17α-hydroxylase activity but no 17,20-lyase activity, resulting in the ultimate conversion of pregnenolone to cortisol. Cells of the *zona reticularis* have both 17α-hydroxylase activity and 17,20-lyase activity, resulting in the conversion of pregnenolone to sex hormone precursors. The abundance of electron donors and presence of cofactors such as CYB5 influence whether the 17,20-lyase reaction will occur. This reaction is upregulated when the *zona reticularis* develops at the time of adrenarche, resulting in the production of weak adrenal androgens such as DHEA. After puberty, approximately half of all circulating testosterone is derived from the adrenal gland in women (and half from the ovary), whereas in men the contribution of adrenal androgens to circulating testosterone is minimal compared with testicular synthesis.

In humans, P450c17 is encoded by a single gene on chromosome 10q24.3. Complete loss of this gene or protein function causes 17α-hydroxylase deficiency with impaired glucocorticoid and sex steroid production and an excess of steroids in the mineralocorticoid pathway. The classic scenario is a phenotypic girl (either 46,XX or 46,XY) who has absent puberty and who is found to be hypertensive and hypokalaemic. Partial defects in P450c17 can present with hypospadias and variable biochemical profiles. Very rarely, specific point mutations in P450c17 can disrupt just the 17,20-lyase activity of the enzyme, resulting in children with impaired sex hormone synthesis (in the gonad, as well as in the adrenal gland).

Electron Transport to P450c17: P450 Oxidoreductase and Cytochrome b5

All *microsomal* forms of cytochrome P450, including P450c17 and P450c21, receive electrons from a membrane-bound flavoprotein, POR (Figure 9.7). This protein is different to the *mitochondrial* flavoprotein adrenodoxin reductase described above.

POR receives two electrons from NADPH and transfers them one at a time to the relevant P450. Because the adrenal endoplasmic reticulum contains many more molecules of P450c17 and of P450c21 than of POR, the P450s compete with one another for the reducing equivalents provided by the reductase. As stated above, the availability of electrons determines whether P450c17 performs only 17α-hydroxylation or also performs 17,20 bond cleavage. The electron transfer necessary for the lyase reaction of P450c17 is promoted by high concentrations of POR and by the action of the allosteric factor CYB5. 17,20-Lyase activity also requires the phosphorylation of serine residues on P450c17 by a cyclic adenosine monophosphate (cAMP)-dependent protein kinase.

Defects in POR (encoded by the gene *POR*) cause combined 17α-hydroxylase and 21-OHD, often in combination with skeletal features as part of Antley–Bixler syndrome. Disruption of CYB5 (encoded by the gene *CYB5A*) affects adrenal and gonadal androgen production and is associated with methaemoglobinaemia.

P450c21 (21-Hydroxylase)

The enzyme P450c21 (21-hydroxylase) is involved in steroid hydroxylation at the #c21 position (Figure 9.5). This reaction can generate 11-deoxycorticosterone (DOC) from progesterone and 11-deoxycortisol from 17-hydroxyprogesterone (17-OHP) (Figure 9.6). Defects in the *CYP21A2* gene cause 21-hydroxylase deficiency (21-OHD), which accounts for more than 90% of cases of congenital adrenal hyperplasia (CAH). A block in 21-hydroxylation causes decreased cortisol and aldosterone synthesis that leads to sodium loss (hyponatraemia), a rise in potassium (hyperkalaemia) and hypotension, with associated cardiovascular collapse and death in the first weeks after birth if not treated appropriately.

In addition to these effects on mineralocorticoids and glucocorticoids, 21-OHD leads to hyperandrogenism both in the early stages of fetal development and postnatally if not treated because decreased synthesis of cortisol and reduced central feedback leads to overproduction of ACTH and overstimulation of the adrenal gland. Since the 21-hydroxylase step is impaired, there is an accumulation of 17-OHP and 17-hydroxypregnenolone. Only a very small amount of 17-OHP is converted to androstenedione by P450c17 but high concentrations of 17-hydroxypregnenolone can undergo conversion to DHEA and subsequently to androstenedione, testosterone and 11-oxygenated androgens (Figure 9.6). This results in prenatal virilization of female (46,XX) fetuses and early puberty in untreated boys and girls.

The P450c21 protein is encoded by the gene *CYP21A2* on chromosome 6p21. This gene and a neighbouring pseudogene are in the middle of the major histocompatibility locus and so 21-OHD can be linked to specific human leucocyte antigen (HLA) types. The P450c21 enzyme is localized to the smooth endoplasmic reticulum and requires POR to transport electrons from NADPH.

P450c11β and P450c11AS (11β-Hydroxylase and Aldosterone Synthase)

Two closely related enzymes, P450c11β and P450c11AS, catalyse the final steps in both glucocorticoid and mineralocorticoid syntheses. These two isozymes have 93% identity in their amino acid sequence and are encoded by tandem duplicated genes on chromosome 8q24.3. The genes are known as *CYP11B1* and *CYP11B2*, respectively.

The two isoforms of P450c11 are localized on the inner mitochondrial membrane and use adrenodoxin and adrenodoxin reductase to receive electrons from NADPH, in a similar manner to P450scc. P450c11β is the most abundant of the two enzymes and is present in both the *zona glomerulosa* and the *zona reticularis*. P450c11β has classic 11β-hydroxylase activity and converts 11-deoxycortisol to cortisol and DOC to corticosterone (Figure 9.6). P450c11AS is the less abundant isoform and is found only in the *zona glomerulosa* where it has 11β-hydroxylase, 18-hydroxylase and 18-methyl oxidase (aldosterone synthase) activities. P450c11AS is thus the key enzyme involved in mineralocorticoid synthesis because it catalyses all the reactions needed to convert DOC to aldosterone.

Defects in *CYP11B1* cause 11β-hydroxylase deficiency. This condition is associated with low cortisol and variable mineralocorticoid production. The phenotype can be modified by the concentration and type of intermediate steroid. Thus, patients often do not have severe effects of hypocortisolaemia but do develop hypertension with time due to very high concentrations of DOC. Hyperandrogenism resulting in virilization of 46,XX fetuses and/or early puberty is usually present due to impaired central feedback and ACTH-dependent generation of androgen precursors, like the phenomenon seen with 21-OHD. By contrast, defects in *CYP11B2* cause aldosterone synthase deficiency and predominantly affect mineralocorticoid production and salt retention.

17β-Hydroxysteroid Dehydrogenase

The 17β-HSD group of enzymes is involved in diverse functions, including oxidation reactions, reduction reactions and other biological effects. They are encoded by distinct genes and can modulate bidirectional reactions in some situations, depending on cofactor availability.

Several 17β-HSDs have key roles in the adrenal glands, gonads and placenta. For example, 17β-HSD type I (17β-HSD1) is involved in the synthesis of oestriol in the placental syncytiotrophoblast cells and in the production of oestradiol in the granulosa cells of the ovary. In contrast, 17β-HSD type II (17β-HSD2) uses NAD^+ to inactivate oestradiol to oestrone and testosterone to Δ^4 androstenedione. This enzyme is expressed in several tissues, including in the endothelial cells of the placental intravillous vessels, where it 'protects' the fetus from the transplacental passage of maternal oestradiol or testosterone.

One of the most important enzymes in this group is 17β-HSD type III (17β-HSD3). This enzyme plays a key role in the conversion of androstenedione to testosterone in the testis (Figure 9.6). Defects in the gene encoding this enzyme, *HSD17B3*, disrupt androgen production and cause 17β-HSD3 deficiency.

17β-HSD type V (17β-HSD4), also known as 3α-hydroxysteroid dehydrogenase, catalyses the reduction of Δ^4 androstenedione to testosterone. It is widely expressed in peripheral tissues and is probably the enzyme responsible for peripheral conversion of androstenedione to testosterone and of DHEA to androstenediol. This enzyme may play a role in the backdoor pathway of androgen (dihydrotestosterone [DHT]) production (see below).

Steroid Sulphotransferase and Sulphatase

Steroid sulphates may be synthesized directly from cholesterol sulphate or may be formed by sulphation of steroids by cytosolic sulphotransferases. The principal sulphotransferase of the adrenal is SULT2A1. This enzyme is especially highly expressed in the fetal adrenal zone during development, as well as in the *zona reticularis* after adrenarche. SULT2A1 sulphates the 3β-hydroxyl group of pregnenolone, 17OH-pregnenolone, DHEA and androsterone. This reaction generates a large pool of less biologically active steroid and protects the fetus from the potentially androgenic effects of DHEA and its metabolites (Figure 9.6). No human mutations in SULT2A1 have been reported but defects in the sulphate donor system necessary to drive the reaction have been (Figure 9.8). Specifically, loss of 3′-phosphoadenosine-5′-phosphosulphate synthase 2 (PAPSS2) results in reduced sulphotransferase activity, causing an increased DHEA/DHEA-S ratio, hyperandrogenism/premature pubarche and skeletal effects (brachyolmia type 4 with epiphyseal and metaphyseal changes) due to altered sulphation of chondroitin in the growth plate cartilage.

The reverse reaction, involving hydrolysation of the steroid sulphate to the native steroid, is catalysed by steroid sulphatase (Figure 9.8). Defects in this enzyme result in reduced availability of free DHEA, a precursor for androgen production and oestrogen synthesis in the placenta. The gene encoding this enzyme is located on chromosome Xp22.3 and is deleted in X-linked ichthyosis. This condition probably results from the accumulation of steroid sulphates in the stratum corneum of the skin.

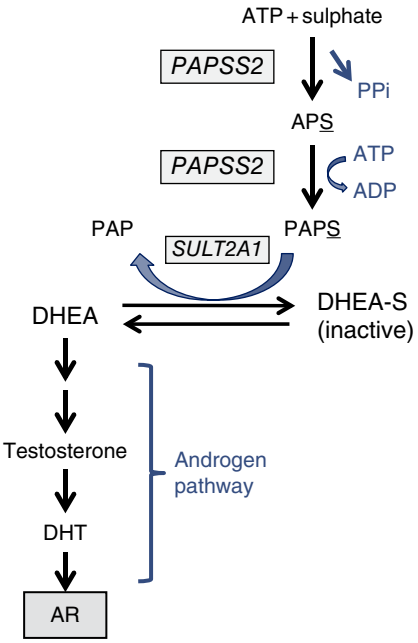


Figure 9.8 The role of PAPSS2 and sulphotransferase in the conversion of DHEA to inactive DHEA-S. PAPSS2 has successive ATP sulphurylase and APS kinase activities. Loss of PAPSS2 activity results in an increased ratio of DHEA to DHEA-S and increased shunting of steroids into the androgen pathway, resulting in hyperandrogenism. PAPSS2 is also important in chondroitin sulphation in cartilage.

P450aro (Aromatase)

All oestrogens (C18 steroids) are generated by the aromatization of androgens (C19 steroids) by a single enzyme P450aro (aromatase) that is encoded by *CYP19A1*, a gene on chromosome 15q21.1, expressed in several key tissues such as the ovary, placenta, bone, adipose tissue and brain. Aromatase expression in each tissue is controlled by different promoters and regulatory regions, as well as by alternatively spliced forms of the enzyme.

Aromatization of fetal adrenal androgens by the placenta is important in the generation of oestrogens in the maternal circulation, as well as for protecting the developing fetus and mother from the effects of high concentrations of adrenal androgens. Defects in aromatase can therefore present with classic features such as progressive maternal virilization towards end of pregnancy, virilization of female (46,XX) infants and features of oestrogen deficiency, such as delayed epiphyseal fusion, osteopenia, absent puberty in girls and dyslipidaemia. Aromatase also converts adrenal androgens to oestrogens in adults but the molar concentrations of these hormones are very small compared to the much greater effects of gonadal steroids.

5α-Reductase

Although testosterone can have a direct effect on the androgen receptor (AR) (NR3C4), the related hormone DHT is a more potent androgen. Testosterone is converted to DHT by the enzyme 5α-reductase in target tissues (Figure 9.6). There are two distinct forms of 5α-reductase, types I and II, encoded by different genes (*SRD5A1* and *SRD5A2*, respectively). The fetal genitals, genital skin and prostate express 5α-reductase type II and this enzyme plays a key role in phallic growth and scrotal development. The type I enzyme is expressed at higher levels after puberty, especially in skin with androgen-dependent hair. Defects in *SRD5A2* cause 5α-reductase type II deficiency characterized by impaired fetal androgenization but with partial virilization at puberty. The changes at puberty may reflect the activity of the type I enzyme in converting high circulating concentrations of testosterone to DHT.

In the past decade there has been increased interest in a 'backdoor' pathway to DHT synthesis, based on work in the Tammar wallaby as well as the steroid profile of patients with P450 oxidoreductase deficiency (PORD).

Several enzymes such as 5α-reductase type I have been implicated in this alternative route to DHT. The exact contribution of the backdoor pathway to human DHT production remains unclear, as is the potential contribution of different tissues to this process.

11β-Hydroxysteroid Dehydrogenase

Glucocorticoids and mineralocorticoids can both bind to the mineralocorticoid receptor (MR) (NR3C2) with similar affinity and circulating concentrations of cortisol are ~1000-fold higher than those of aldosterone. Therefore, a mechanism is needed to prevent activation of the MR by glucocorticoids in mineralocorticoid-dependent tissues such as the kidney, which would have devastating effects on sodium reabsorption and blood pressure. This effect is provided by the conversion of cortisol to the bioinactive steroid cortisone.

The interconversion of cortisol and cortisone is mediated by two isozymes of 11β-HSD, each of which has both oxidase and reductase activity (Figures 9.6 and 9.9).

The type I enzyme (11β-hydroxysteroid dehydrogenase type I [11β-HSD1], *HSD11B1*) is expressed mainly in glucocorticoid-responsive tissues, such as the liver, testis, lung and proximal convoluted tubule. 11β-HSD1 can catalyse the oxidation of cortisol to cortisone using $NADP^+$ as its cofactor (K_m 1.6 μmol/L) or the reduction of cortisone to cortisol using NADPH as its cofactor (K_m 0.14 μmol/L). The enzyme can only function at high concentrations of the steroid (micromolar). The direction of the reaction is influenced by substrate concentration and the availability of cofactor.

The type II enzyme (11β-hydroxysteroid dehydrogenase type II [11β-HSD2], *HSD11B2*) can function with low (nanomolar) concentrations of steroid (K_m 10–100 nmol/L). This enzyme catalyses only the oxidation of cortisol to cortisone using NAD. 11β-HSD2 is expressed in mineralocorticoid-responsive tissues and therefore prevents activation of the MR by cortisol. This enzyme does not catalyse 'true' mineralocorticoids such as aldosterone, so the salt-retaining effects of aldosterone are unaffected.

In the placenta and other fetal tissues, 11β-HSD1 also inactivates cortisol. The placenta has abundant $NADP^+$, which supports the oxidative action of 11β-HSD1. Therefore, both enzymes protect the fetus from high maternal concentrations of cortisol.

11β-HSD1 is not in contact with the cytoplasm but is located within the endoplasmic reticulum. Here it receives NADPH by the action of hexose-6-phosphate dehydrogenase (H6PD), linking 11β-HSD1 to the pentose monophosphate shunt and energy storage as fat. Disruption of H6PD has been reported in apparent cortisone reductase deficiency (ACRD) (cortisone reductase deficiency [CRD] type I), whereas disruption of 11β-HSD1 has been reported rarely in CRD type II. Both these conditions reduce cortisol feedback and have mild ACTH-driven hyperandrogenism, with classic biochemical profiles especially on urine steroid analysis. Mutations in 11β-HSD2 cause the condition apparent mineralocorticoid excess (AME) [7].

Dynamic Regulation of Steroidogenesis

Overview of the HPA Axis

Cortisol secretion is an example of a classical endocrine feedback loop under negative feedback control from the HPA axis (Figure 9.1) (Chapter 5).

ACTH controls cortisol synthesis and secretion in the adrenal gland and its secretion is stimulated by corticotropin-releasing hormone (CRH) (also known as CRF) from the hypothalamus and follows a circadian pattern. ACTH is also released during periods of stress such as trauma, hypoglycaemia, infection and fever, surgery or anxiety. High circulating concentrations of cortisol or synthetic glucocorticoids inhibit ACTH release. The negative feedback occurs at both the hypothalamic (CRH) and pituitary (ACTH) level (Figure 9.1).

Hypothalamic CRH

CRH, a 41-amino-acid peptide, is the physiological stimulator of ACTH synthesis and secretion and is synthesized by neurons in the hypothalamic paraventricular nucleus, from which it reaches the anterior pituitary target cells via the pituitary portal circulation as described above. The paraventricular nucleus also synthesises arginine vasopressin (AVP) (also known as

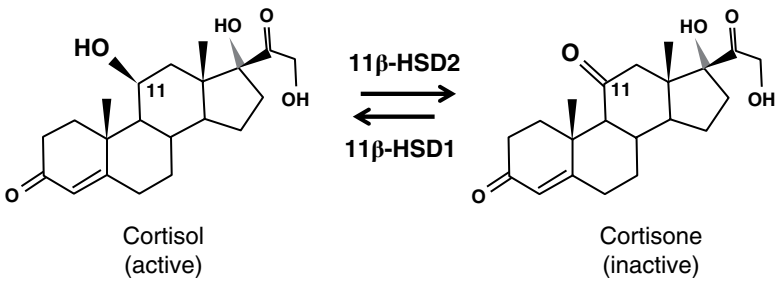


Figure 9.9 The cortisol to cortisone shuttle catalysed by the 11β-hydroxysteroid dehydrogenase enzymes (11β-HSD1 and 11β-HSD2).

antidiuretic hormone [ADH]). Most AVP axons terminate in the posterior pituitary, potentiating the classical renal and vascular effects of AVP. AVP can also stimulate the release of ACTH and potentiate CRH action; it is thought to play a role in the stress response.

CRH and AVP stimulate ACTH secretion through different mechanisms. CRH stimulates ACTH synthesis and release through the protein kinase A pathway, via intracellular cAMP but AVP stimulates intracellular Ca^{2+} through the protein kinase C pathway.

CRH secretion follows a circadian rhythm generated in the suprachiasmatic and paraventricular nuclei of the hypothalamus. Stress-mediated release of ACTH can be superimposed on to this circadian rhythm at any time. The most potent stimulator of CRH secretion is acute inflammation mediated by the inflammatory cytokines. Cortisol has a striking inhibitory effect on the synthesis and secretion of CRH and AVP.

Pituitary (Corticotroph) POMC and ACTH

ACTH is a single-chain 39-amino-acid peptide derived from pro-opiomelanocortin (POMC), which is synthesized in and secreted from pituitary corticotroph cells. POMC is a prohormone that encodes multiple smaller peptide hormones within its structure, including ACTH and α- and β-melanocyte-stimulating hormone (MSH) (Figure 9.10). These biologically active peptides and hormones are generated by cleavage of POMC at basic residue cleavage sites by prohormone-converting enzymes (mostly prohormone convertase 1 [PC1]). The peptides are stored within cells in dense core secretory granules until release.

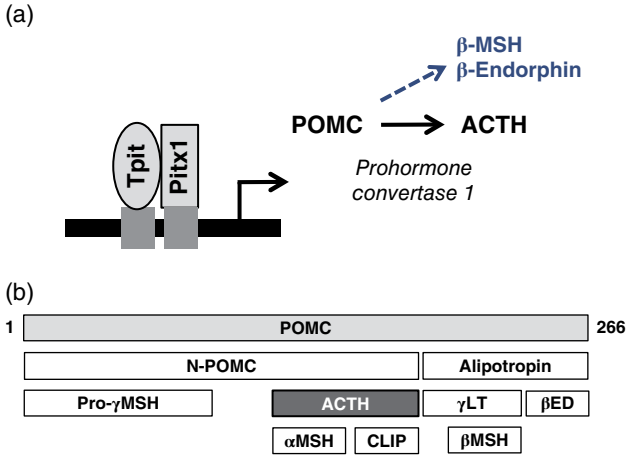


Figure 9.10 Central regulation of ACTH synthesis and release. (a) The role of transcription factors Tpit (*TBX19*) and Pitx1 in corticotroph-specific regulation of pro-opiomelanocortin (POMC). (b) Cleavage of POMC by prohormone convertase 1 generates ACTH and several other important peptide hormones involved in appetite regulation, skin pigmentation and neuronal function.

ACTH (1-39) is derived from POMC residues 138–176. Only the first 20–24 amino acids of ACTH are required for normal biological activity and shorter isoforms of ACTH (such as 1–24; tetracosactide) are used in diagnostic tests to evaluate adrenal function. The other peptides derived from POMC include α-MSH involved in satiety and β-MSH involved in skin/hair pigmentation. POMC cleavage also generates β-endorphin, which is released to activate opioid pathways in the body in response to pain.

The corticotroph cells in the anterior pituitary are primarily under the positive regulation of CRH and negative regulation of glucocorticoids. Tissue-specific transcription factors also play an important role in regulating expression of POMC in different parts of the body. For example, TPIT (*TBX19*) is the key factor controlling POMC expression in the corticotroph cells (Figure 9.10). Consequently, defects in TPIT cause isolated ACTH insufficiency and secondary (central) AI, whereas disruption of POMC itself or of the cleavage PC1 (PCSK1) produce an extended phenotype including obesity, as well as skin/hair or gastrointestinal effects, respectively.

Ectopic ACTH secreted from malignant tumours is derived from ectopic synthesis of the same POMC precursor; however ectopic ACTH secretion is not under the same tight feedback regulation seen in the HPA axis.

ACTH Action on the Adrenal Gland

ACTH is released from the pituitary via the hypophyseal vein and circulates to the adrenal cortex where it elicits both acute and long-term effects. Acute effects to induce steroidogenesis occur within minutes and are mediated by ACTH binding to its specific cell surface receptor, the ACTH receptor (or melanocortin 2 receptor [MC2R]). MC2R is a seven-transmembrane domain G-protein-coupled receptor and part of the melanocortin receptor family that includes MC1R–MC5R. These receptors are involved in diverse biological functions, including adrenal steroidogenesis, pigmentation and weight and energy homeostasis. The sole natural ligand for MC2R is ACTH, in contrast to the other melanocortin receptors that show varying affinity to ACTH and α-, β- and γ-MSH.

ACTH binding to MC2R induces intracellular production of cAMP, one of the major actions of which is to stimulate cAMP-dependent protein kinase (protein kinase A). This in turn upregulates LDL receptor biosynthesis and the uptake of LDL cholesterol into the cell via receptor-mediated endocytosis. The resulting vesicles store cholesterol in the form of cholesterol esters; these fuse with lysozymes allowing hydrolysis of the cholesterol ester by HSL and release of free cholesterol. ACTH also facilitates *de novo* cholesterol synthesis by stimulating acetyl coenzyme A, the rate-limiting step in cholesterol biosynthesis.

The ACTH-mediated intracellular increase in cAMP also stimulates the synthesis and phosphorylation of steroidogenic acute regulatory protein (StAR). Activation of the nuclear transcription factor cAMP response element binding protein (CREB) increases the expression of all steroidogenic enzymes including StAR. StAR protein facilitates transport of intracellular cholesterol from the outer to the inner mitochondrial membrane. Cholesterol can then be converted within the mitochondria to pregnenolone by cytochrome P450scc (CYP11A1) to enter the steroidogenic pathways (Figure 9.6).

Long-term effects of ACTH include increased steroidogenic enzyme expression and accumulation by several mechanisms that include stimulating gene transcription and increased mRNAs. These mechanisms result in an increased rate of cortisol synthesis through effects at each step of the pathway. Prolonged exposure to glucocorticoids inhibits ACTH synthesis by directly inhibiting POMC gene transcription.

ACTH has trophic effects on the adrenal gland and may influence the differentiation of progenitor stem cells. MC2R is highly expressed in the fetal adrenal gland. Absence of ACTH production (due to hypopituitarism or anencephaly) or resistance to its action (e.g. MC2R mutations) can result in hypoplastic adrenal glands, supporting a role for ACTH in adrenal growth. ACTH probably has an ongoing trophic effect on postnatal adrenal size and chronic suppression of ACTH by exogenous steroids can be associated with reduced adrenal size and reduced responsiveness.

Diurnal Regulation of Steroidogenesis

Glucocorticoid concentrations demonstrate a circadian rhythm with robust circadian variation (Figure 9.11). The hypothalamic content of CRH also shows a circadian rhythm with peaks at about 4am, consistent with peak ACTH levels at ~4–6 am. Cortisol concentrations start to rise at ~3 am and reach a peak between 8 and 9 am before gradually falling to a nadir at night with concentrations typically below 50 nmol/L at midnight. This circadian rhythm is generated by an underlying ultradian rhythm of episodic secretory pulses of both ACTH and cortisol every 30–120 minutes; the frequency and amplitude of these pulses are greater in the morning.

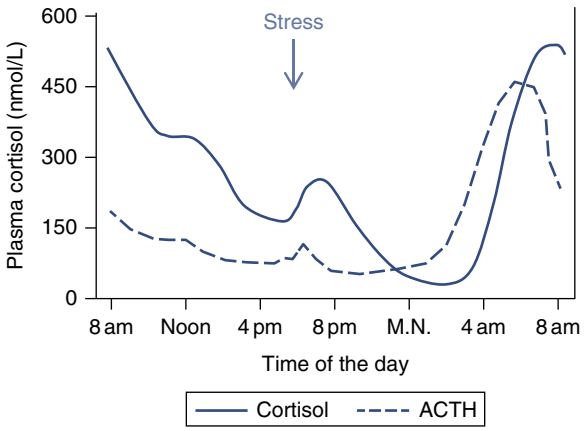


Figure 9.11 Representative profile showing the circadian variation in circulating plasma cortisol concentrations, ACTH (not to scale) and the effect of superimposed stress.

The molecular and cellular basis of the circadian rhythm remains incompletely understood. This phenomenon is regulated primarily by the HPA neuroendocrine axis but multiple regulatory mechanisms within the molecular circadian clock also contribute to the diurnal rhythm.

To adapt to and anticipate external daily cycles, many animals have developed an autonomous and self-sustainable circadian clock that is continuously affected by external factors called 'zeitgeber'. These factors include light–dark cycles and feeding. The suprachiasmatic nucleus (SCN) of the anterior hypothalamus functions as the master circadian clock. The SCN drives key circadian rhythms, including the secretion of hormones such as glucocorticoids, via the HPA neuroendocrine axis and the autonomic nervous system via sympathetic splanchnic innervation of the adrenal gland. The circadian rhythm generated in the SCN coordinates and communicates with the various peripheral clocks throughout the body.

'Clock genes' are required for the generation and maintenance of the circadian rhythm both centrally and within different organs. The adrenal peripheral clock also mediates the daily rhythms of glucocorticoid synthesis, probably by modifying the capacity and responsiveness of adrenal glucocorticoid secretion to ACTH stimulation via StAR. Glucocorticoids in turn influence the synchronization of other peripheral clocks and the regulation of physiological processes including metabolism.

Neonates do not have a circadian rhythm of sleep, feeding or cortisol secretion but acquire behavioural day–night sleep patterns first. Cortisol rhythms are usually established in the first few months of life and, once the rhythm is well established, it is difficult to change. For example, when travelling between time zones, cortisol rhythms can take more than 10 days to adjust.

A growing body of evidence suggests that not only the level of circulating glucocorticoid but also its rhythmic activity plays a significant role in human health and disease. Alterations in glucocorticoid rhythmicity have been implicated in Cushing syndrome (CS), depression, Alzheimer's disease and metabolic syndrome. Abnormalities of the ACTH and cortisol axis often occur in chronic fatigue syndrome, sepsis and post-traumatic stress disorder or with

chronic jet lag or shift work. Further insight into the molecular and cellular basis of the glucocorticoid circadian rhythm is needed to understand the importance of the HPA axis in these conditions [8, 9]).

A stress-mediated response can be superimposed on the circadian rhythm at any time (Figure 9.11). Severe trauma, major surgery, high fever or serious illness can increase the secretion of ACTH and cortisol through activation of the HPA axis but minor illnesses have little effect. The most potent stimulator of CRH secretion is acute inflammation mediated by the inflammatory cytokines (e.g. IL-1, IL-6 and tumour necrosis factor).

Glucocorticoid Feedback

The HPA axis is a classic endocrine feedback system (Figure 9.1). CRH stimulates ACTH, which in turn stimulates cortisol secretion. Cortisol maintains equilibrium by directly acting not only on the pituitary to inhibit ACTH secretion but also on the hypothalamus to inhibit CRH secretion, consequently limiting the amount of cortisol released acutely into the circulation. In more chronic situations, cortisol has an effect by downregulating the transcription of key genes such as POMC, as well as through a hippocampal feedback loop. Other adrenal cortex hormones stimulated by ACTH secretion such as adrenal androgens do not exert regulatory feedback on ACTH secretion.

Aldosterone Production and the Renin–Angiotensin System

Aldosterone has the most potent mineralocorticoid activity of all naturally occurring steroids. It is synthesized from cholesterol in the *zona glomerulosa* (Figure 9.6) and causes renal sodium retention and potassium loss consequently increasing intravascular volume and blood pressure. The control of mineralocorticoid production is largely separate from glucocorticoid production. The main factor controlling aldosterone production is angiotensin through the renin–angiotensin–aldosterone system (RAAS) (Figure 9.12). High concentrations of ACTH can also stimulate aldosterone release acutely, although chronic pharmacological doses of ACTH supress aldosterone. The relative independence of the mineralocorticoid and glucocorticoid systems is clinically important as some patients may have disorders affecting only one system or may have combined glucocorticoid and mineralocorticoid deficiency due to adrenal hypoplasia or destruction.

Renin synthesis occurs from the juxtaglomerular epithelial cells of the renal tubule in the kidney in response to low renal arteriolar pressure, hyperkalaemia, hyponatraemia, upright posture, vasodilatory drugs and β-adrenergic stimulation (Figure 9.12) but the system is primarily regulated by body sodium and potassium status, potassium being a potent direct stimulus of aldosterone secretion (Figure 9.1). Circulating aldosterone concentrations show diurnal variation that mirrors the pattern shown by cortisol but fluctuations are superimposed on this pattern depending on electrolyte balance and postural changes.

Renin secretion is the rate-limiting step within the RAAS and is controlled through a negative feedback loop. Renin is a serine protease enzyme synthesized as a precursor that is cleaved initially to prorenin and then to the 340-amino-acid protein found in plasma. Renin cleaves the N-terminal 10 amino acids of angiotensinogen to form angiotensin I (Figure 9.12). Angiotensinogen-converting enzyme (ACE), found primarily in blood vessels and lungs, subsequently cleaves off the two carboxy-terminal (C-terminal) amino acids of angiotensin I to form the active octapeptide angiotensin II.

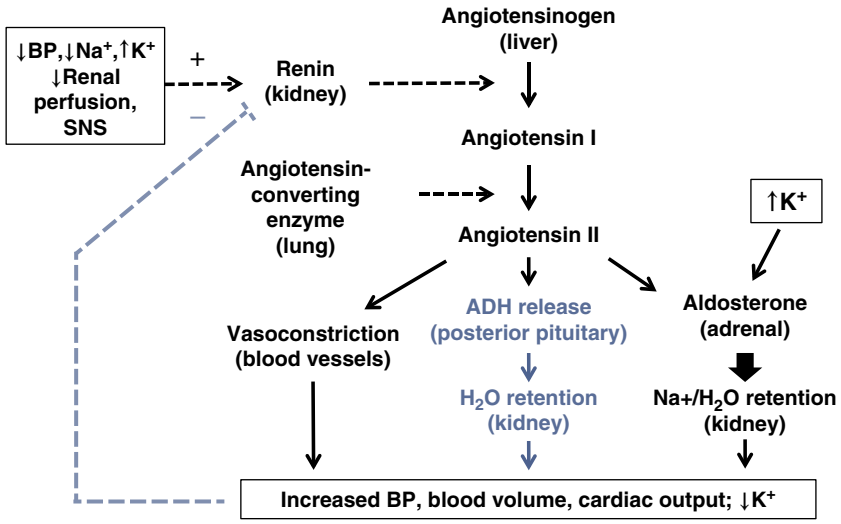


Figure 9.12 Overview of the renin–angiotensin system (RAAS) and regulation of aldosterone release. BP – Blood Pressure, SNS – Sympathetic Nervous System.

Angiotensin II has two principal actions, both of which are mediated through the AT_1 receptor and increase blood pressure. Angiotensin II stimulates arteriolar vasoconstriction within seconds but it is also a potent stimulator of aldosterone secretion by increasing transcription of the cytochrome P450 enzyme CYP11B2 (aldosterone synthase) within the *zona glomerulosa* cells of the adrenal cortex. Increased plasma potassium is a powerful stimulator of aldosterone production via membrane depolarization, calcium channel alteration and ultimately increased transcription of CYP11B2. Both angiotensin II and potassium work at different levels of the same intracellular second messenger pathway but differ fundamentally from the action of ACTH. Angiotensin II may also influence AVP release.

Several additional factors can influence aldosterone secretion. Atrial natriuretic peptide (ANP) and dopamine inhibit aldosterone secretion, whereas adrenaline, noradrenaline and vasoactive intestinal peptide all stimulate aldosterone secretion through different mechanisms [10].

Adrenal Androgen Production and the Regulation of Adrenarche

The adrenal cortex produces the weak androgen DHEA that is regulated by ACTH so DHEA concentrations reach their peak in the morning. DHEA secretion shows marked differences across the lifespan primarily related to the development of the adrenal gland and its zones.

The fetal adrenal secretes large amounts of DHEA and DHEA-S from the FZ and these steroids are abundant in the newborn. Their concentrations fall rapidly as the FZ involutes after birth and then the adrenal gland secretes minimal quantities of adrenal androgen precursors until the onset of adrenarche, usually around the age of 7–8 years and preceding the onset of puberty by about 2 years. The rise in the circulating concentrations of DHEA and DHEA-S is the biochemical hallmark of adrenarche.

Adrenarche coincides with the development of the *zona reticularis* and is independent of puberty, the gonads and gonadotropins. ACTH has a permissive role in adrenarche but does not trigger it. The exact mechanisms regulating the development of the *zona reticularis* and adrenal androgen secretion at adrenarche remain poorly understood.

Several hypotheses have been proposed to explain the development of the *zona reticularis*. One theory is that the differentiation of cells into zones is regulated by the gradient of a morphogen such as hedgehog, Wnt or Notch. This hypothesis is consistent with the observed cell migration model involved in adrenal development and maintenance (Figure 9.3). Indeed, lineage tracing studies in the mouse have shown that all adrenal cortical cells are derived from *zona glomerulosa* precursors that migrate centrally and differentiate into different cell types. It is proposed that the morphogen would be at its highest concentration near the capsule and reducing to a low level adjacent to the medulla.

A second hypothesis proposes that a specific hormone may act on a precursor cell (either a stem cell or *zona fasciculata* cell) to cause development of the *zona reticularis*.

The roles of 3β-HSD2 (HSD3B2), CYB5 and SULT2A1 have been studied in detail. HSD3B2 expression appears to decrease with the onset of adrenarche and the adrenal expression of CYB5, which mediates the 17,20-lyase activity of P450c17, is confined almost exclusively to the *zona reticularis*. The differential expression of these proteins appears to be critical for reticularis function and the mechanisms regulating the transcription of these genes may provide insight into the development of the adrenal reticularis and its function.

The role of the adrenal *zona reticularis*, adrenarche and adrenal androgen synthesis is becoming more important as several studies have indicated that children with premature adrenarche may be at increased risk of polycystic ovary syndrome (PCOS), increased body mass index (BMI) and metabolic syndrome in later life. DHEA is an important precursor of sex steroid synthesis in women and generates ~50% of circulating androgens. Although DHEA deficiency causes androgen deficiency in females, many additional functions distinct from its role in sex hormone production have been proposed [11, 12].

For example, DHEA concentrations have been associated with age-related changes in cardiovascular function, female fertility, metabolism and central nervous system (CNS) function. DHEA-S can cross the blood–brain barrier and may play a role in human brain maturation. DHEA can also potentially be synthesized in the brain directly by *de novo* steroidogenesis. Some studies have suggested that declining DHEA and DHEA-S levels are associated with age-related disorders but the benefit of DHEA replacement in women with primary AI (PAI) or with age remains controversial.

Steroid Hormone Actions

Circulation of Steroid Hormones

More than 50 different steroids have been isolated from adrenocortical tissue. Most are not components of steroidogenic pathways and only a few are secreted in significant quantities. Cortisol is secreted in high concentrations (~10–20 mg/day in adults) compared with aldosterone, which is secreted in low amounts (~100–150 μg/day). The adrenal androgen precursors – DHEA, its sulphate ester DHEA-S and androstenedione – are the most abundant steroids secreted by the

Table 9.2 Potency of selected therapeutic steroids compared with cortisol based on their *anti-inflammatory* effect.

Steroid	Anti-inflammatory effect	Growth-restricting effect	Salt-retaining effect	Plasma half-life (min)	Biological half-life (h)
Cortisol (hydrocortisone)	1.0	1.0	1.0	80–120	8
Cortisone acetate (oral)	0.8	0.8	0.8	80–120	8
Cortisone acetate (IM)	0.8	1.3	0.8		18
Prednisone	3.5–4	5	0.8	200	16–36
Prednisolone	4		0.8	120–300	16–36
Methylprednisolone	5	7.5	0.5		
Betamethasone	25–30		0	130–330	
Dexamethasone	30	80	0	150–300	36–54
9α-Fludrocortisone	15		200		
Aldosterone	0.3		200–1000		

adult adrenal gland (more than 20 mg daily). Therefore, although cortisol and aldosterone are both essential for life, they are secreted in significantly different quantities with a 100- to 1000-fold molar difference. This is important when considering the impact of steroid-binding proteins and incomplete defects in steroidogenesis.

Most circulating cortisol (>90%) is bound to plasma proteins, predominantly corticosteroid-binding globulin (CBG), with a small amount bound to other binding proteins including albumin and α_1-acid glycoprotein. CBG is a 383-amino-acid protein synthesized in the liver that binds cortisol with a high affinity. The steroid-binding proteins are not transport proteins but act as a reservoir for steroids ensuring that all peripheral tissues are exposed to approximately equal quantities of cortisol and buffering the physiological effect of diurnal variation.

Circulating CBG concentrations are significantly increased by oestrogens and reduced in patients with cirrhosis, renal impairment and hyperthyroidism. The oestrogen effect should be taken into consideration if measuring total cortisol concentrations in pregnant women or patients on oestrogen replacement therapy. Very rare inherited conditions can also affect CBG concentrations but in these conditions free cortisol levels are normal.

Aldosterone is weakly bound to plasma proteins and therefore has a relatively short half-life of about 20 minutes and a high metabolic clearance rate. Changes in plasma protein concentration do not affect plasma aldosterone concentrations in contrast to cortisol concentrations, which are greatly influenced by changes in protein concentrations. Oestrogen and testosterone bind strongly to a different binding protein, sex hormone-binding globulin, and weakly to albumin.

Synthetic glucocorticoids do not bind significantly to cortisol-binding proteins, except for prednisolone that has an affinity of ~50% that of cortisol. This lack of binding results in greater bioavailability and greater potency, which is increased further by the increased receptor binding affinities of most synthetic steroids (Table 9.2).

The concentration of free or unbound circulating steroids is obviously an important factor in determining biological activity but the target tissues for many of these hormones contain enzymes that modify this. For example, 11β-HSD2 can inactivate cortisol to cortisone; 5α-reductase type 2 converts testosterone to the more active metabolite DHT; there are 'extra-adrenal' enzymes such as 21-hydroxylase, P450 aromatase, 3β-HSD and members of the 17β-HSD family, which allow peripheral metabolism of steroids. Thus, steroids can act as classic hormones and as precursors to generate locally acting autocrine or paracrine factors.

Steroid Hormone Receptors

Cortisol and aldosterone exert their effects through binding of free hormone to intracellular receptors termed glucocorticoid and mineralocorticoid receptors, respectively. These proteins are members of the nuclear receptor superfamily of transcription factors and are officially named NR3C1 and NR3C2. Both receptors have a C-terminal ligand-binding domain (LBD), a central DNA-binding domain (DBD) and a variable domain at the N-terminal region. The LBD consists of a 12-helix structure that binds hormone and recruits co-activators. The DBD interacts with specific DNA sequences on target genes in the nucleus and results in increased (or sometimes decreased) gene transcription [5].

Glucocorticoid Receptor (GRα, NR3C1)

The glucocorticoid receptor (GR) (glucocorticoid receptor α [GRα]) is present in the cytoplasm of almost all cells

and while inactivated is surrounded by numerous chaperone proteins. Cortisol passively diffuses through the cell membrane to activate the GR, causing a conformational change and dissociation of the heat shock proteins (HSP) from the receptor. This process allows the GR to translocate to the nucleus (Figure 9.13).

There are two major modes of GR action: the *classic* pathway involves stimulation or repression of gene transcription following binding of the GR to positive or negative glucocorticoid-responsive elements in the target gene and the recruitment of co-activator complexes. *Non-classic* mechanisms of action include direct actions through the interaction with proteins or non-genomic signalling pathways through cell surface binding.

Glucocorticoids exert widespread effects throughout the body and control many physiological processes. At least 10% of all genes are influenced by glucocorticoid concentrations. Glucocorticoids increase gluconeogenesis, proteolysis and lipolysis and modulate lipid deposition. They also regulate numerous immune and inflammatory responses and elicit both positive and negative effects on cell growth. Glucocorticoids can influence mood, behaviour and cognition and elevated concentrations increase depression and anxiety. They increase blood pressure by increasing cardiovascular tone and act on both neuronal and glial cells in the CNS to influence cognition, organizational events in the developing brain and neuronal degeneration in adulthood. Many of the systemic symptoms and signs seen in patients with AI or adrenal excess can be explained by these widespread actions.

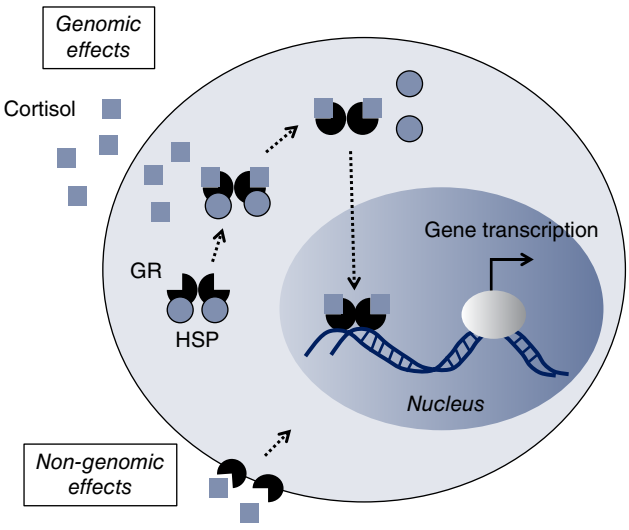


Figure 9.13 Cartoon showing the classic cellular actions of cortisol on gene transcription in the nucleus (upper pathway, genomic effects), as well as possible non-genomic actions (lower pathway). GR, glucocorticoid receptor; HSP, heat shock proteins.

Mineralocorticoid Receptor (MR, NR3C2)

Aldosterone acts both genomically and non-genomically primarily through the MR (NR3C2). The classical genomic effects of aldosterone are mediated through aldosterone binding to the C-terminal domain of the MR causing a conformational change that allows the receptor to translocate to the cell nucleus where it binds to aldosterone-responsive genes to activate or repress gene transcription.

One of the most important physiological actions of aldosterone is to increase sodium reabsorption through the kidney and through other epithelial sites. Aldosterone acts to increase the expression of the epithelial sodium channel (ENaC), increasing the number of channels and ensuring that they remain open to allow the passage of Na+. The ENaC is located in the renal distal convoluted tubule and facilitates reabsorption of sodium in exchange for potassium and hydrogen ions (Figure 9.12). Aldosterone has a profound effect on acid–base balance as it additionally regulates hydrogen ion excretion through a sodium-insensitive route in the intercalated cells of the collecting tubule in the distal nephron. It acts to promote hydrogen ion excretion through regulation of the ATP-dependent apical hydrogen ion pump and the basolateral membrane Cl^-/HCO^3 exchanger. Aldosterone may also increase blood pressure through direct vasoconstrictor effects of MR activation on the vascular wall, through cardiac effects and through CNS-mediated events.

Aldosterone was previously thought to be the only physiological ligand for the MR but cortisol, corticosterone and DOC can all bind to the receptor and activate its function. Cortisol concentrations are ~100-fold higher than those of aldosterone and inactivation of cortisol to cortisone by the enzyme 11β-HSD2 in the epithelial target cells 'protects' the receptor from the potentially devastating effects of cortisol action (Figure 9.9).

Mineralocorticoid sensitivity varies considerably throughout childhood and into adult life. Newborn babies and infants have relative mineralocorticoid resistance so aldosterone concentrations are very high after birth and fall in the first few years of life (Figure 9.14). Plasma renin activity (PRA) shows a similar pattern (Figure 9.14). Thus it is important to use appropriate pediatric reference ranges when assessing the RAAS in infants and young children.

Androgen Receptor (AR, NR3C4)

DHEA and DHEA-S are the most abundant endogenous circulating steroid hormones. DHEA, DHEA-S and androstenedione are secreted almost exclusively by the *zona reticularis*. These steroids are referred to as adrenal androgens because they can be converted peripherally to

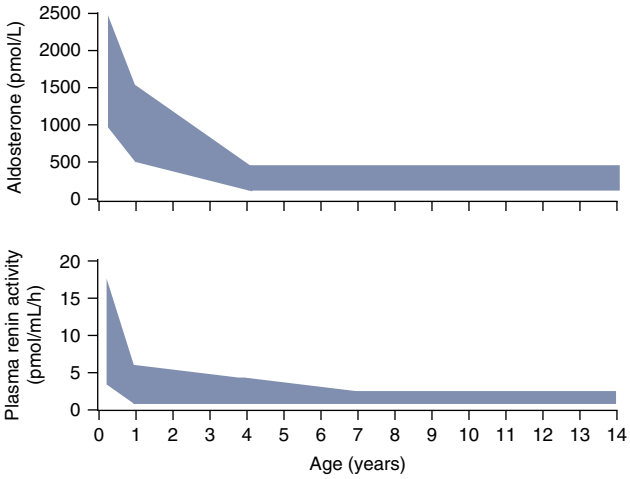


Figure 9.14 Typical normal ranges for aldosterone and plasma renin activity in childhood and young adulthood.

testosterone, which in turn can act on the AR to mediate an effect on hair growth (especially pubic and axillary hair), sweat and sebum production in the skin and hair follicle and potentially libido. DHEA and DHEA-S have little if any capacity to bind to and activate AR and are therefore androgen precursors but not true androgens. A small amount of androgen generated from DHEA is converted to oestrogens, such as oestradiol. The relative contribution of this to total oestrogen in premenopausal women is small but in postmenopausal women, over 90% of oestradiol is from adrenal DHEA conversion to oestrogens in adipose tissue.

In addition to being metabolized to active sex hormones, DHEA can act directly on many hepatic nuclear receptors such as PPARα, which regulates transcription of CYP genes, constitutive androstane receptor (CAR) and PXR. DHEA can activate G-protein-coupled receptors and mediate acute cell signalling pathways. It increases bone mineral density in men and women by increasing IGF-1 gene transcription. DHEA and DHEA-S have been shown to act in neuronal and CNS cells and there is great interest in their effects in cognitive function. The potential benefit of DHEA and DHEA-S in cardiovascular disease has been postulated in many studies but remains controversial. The potential effects of DHEA and DHEA-S over and above their roles as androgen precursors are intriguing.

Steroid Hormone Catabolism

Approximately 1% of circulating cortisol and aldosterone is excreted directly through the kidneys. In the case of cortisol this is termed urinary free cortisol (UFC). The liver metabolizes most circulating steroid, rendering it more soluble and therefore more easily excretable by the kidneys. Many hepatic metabolites of each steroid are produced; most contain additional hydroxyl groups and are linked to a sulphate or glucuronide moiety.

The various urinary metabolites produced can be measured by mass spectrometry so that a pooled 24 hour urine sample can be used to study adrenal steroid production *in vivo* and identify potential enzyme deficiencies and adrenal conditions. Approximately 50% of cortisol is secreted as tetrahydrocortisol (THF), 5α-tetrahydrocortisol (allo-THF) and tetrahydrocortisone (THE), 25% as cortols/cortolones, 10% as #c19 steroids and 10% as cortolic/cortolonic acids.

Aldosterone is excreted primarily through the liver with ~40% being converted to tetrahydroaldosterone before being further metabolized in the kidney prior to excretion. Only a small percentage is excreted directly in the urine.

Adrenal Insufficiency (AI)

Overview of AI

AI is a rare condition associated with considerable mortality and morbidity unless it is diagnosed and treated appropriately.

Primary AI results from defects in the adrenal gland itself. It affects 1:8,000–10,000 children but it is possible that many more newborns and children die undiagnosed, especially in places where there is limited access to healthcare and where sepsis or other causes of childhood mortality are high.

Secondary (or central) AI results from defects in the hypothalamic–pituitary system that disrupt ACTH secretion. ACTH secretion is relatively robust compared with other pituitary hormones and is usually the last hormone to be affected in conditions such as multiple pituitary hormone deficiency or postcranial irradiation. Isolated central ACTH deficiency does occur.

Iatrogenic causes of AI, when the HPA axis is suppressed by exogenous steroid treatment, occur in a significant proportion of children receiving steroids for many medical conditions such as renal disease, inflammatory conditions and asthma. All healthcare professionals looking after such children need to be aware of the risks of steroid treatment, especially during illness and stress and following weaning. Caregivers and young people need to be educated appropriately.

AI can be caused by many underlying conditions. An overview of the main causes is shown in Table 9.3, loosely based on the International Classification of Pediatric Endocrine Diagnoses (www.icped.org). The relative prevalence of these conditions needs attention: 21-OHD is common compared to other steroidogenic defects and should be top of the differential diagnosis in a newborn

Table 9.3 Causes of adrenal insufficiency.

Secondary adrenal insufficiency
- Isolated ACTH deficiency
  - POMC deficiency
  - Prohormone convertase 1 (PC1) deficiency
  - TPIT (TBX19)
- Congenital multiple pituitary hormone deficiency
  - Genetic causes: HESX1, GLI2, OTX2, SOX3, LHX4 (LHX3, PROP1; delayed onset), AD GHD type 2 due to *GH1* mutation
  - Idiopathic (unknown)
- Acquired multiple pituitary hormone deficiency
  - Infiltrative/inflammatory disorders (e.g. LCH, sarcoidosis, haemachromatosis)
  - Postcranial irradiation
  - Post-surgical
  - Post-trauma
  - Tumours (e.g. pituitary, craniopharyngioma)
- ACTH suppression
  - Chronic infection (e.g. HIV)
  - Chronic stress
  - Glucocorticoid suppression (iatrogenic)

Primary adrenal insufficiency
- Adrenal hypoplasia
  - X-linked adrenal hypoplasia congenita (AHC) (NR0B1/DAX-1)
  - Steroidogenic factor 1 related (NR5A1/SF-1)
  - IMAGe syndrome (CDKN1C, also POLE1)
  - MIRAGE syndrome (SAMD9)
  - SERKAL syndrome (WNT4)
  - Idiopathic (unknown)
- Familial glucocorticoid deficiency (FGD)-like conditions (ACTH resistance)
  - MC2R (ACTH receptor) (FGD1)
  - MRAP (FGD2)
  - Non-classic STAR (FGD3)
  - Non-classic CYP11A1
  - Nicotinamide nucleotide transhydrogenase (NNT)
  - Thioredoxin reductase 2 (TXNRD2)
  - Minichromosome maintenance 4 (MCM4)
  - Triple A syndrome
  - Sphingosine-1-phosphate lyase 1(SGPL1)
- Congenital adrenal hyperplasia
  - Congenital lipoid adrenal hyperplasia (*STAR*)
  - P450scc (CYP11A1)
  - 3β-Hydroxysteroid dehydrogenase type II (HSD3B2)
  - 21-Hydroxylase (CYP21A2)
    - Salt wasting
    - Simple virilizing
    - Non-classic
  - 11β-Hydroxylase (CYP11B1)
  - 17α-Hydroxylase/17,20-lyase (CYP17A1)
  - P450 oxidoreductase (POR)
- Autoimmune adrenalitis (Addison disease)
  - Autoimmune polyglandular syndrome type 1 (APECED) (AIRE)
  - Autoimmune polyglandular syndrome type 2
  - Isolated Addison disease
- Metabolic causes
  - Smith–Lemli–Opitz syndrome
  - Adrenoleukodystrophy/adrenomyeloneuropathy
  - Neonatal adrenoleukodystrophy
  - Primary xanthomatosis (Wolman disease)
  - Mitochondrial disorders
  - Other
- Glucocorticoid resistance (GR, NR3C1)
- Disorders of aldosterone synthesis and action
  - Aldosterone synthase (CYP11B2)
  - Mineralocorticoid resistance (pseudohypoaldosteronism type 1) (MR, NR3C2)

Table 9.3 (Continued)

Infections
Tuberculosis
Fungal infections (histoplasmosis, coccidioidomycosis)
Bacterial sepsis (meningococcal, pneumococcal, streptococcal, haemophilus)
HIV associated
Haemorrhage
Associated with meningococcal infection (Waterhouse–Friderichsen syndrome)
Trauma
Idiopathic
Infiltrative
Metastatic disease
Amyloidosis, sarcoidosis, haemochromatosis
Drug effects
Idiopathic (unknown)

male presenting with a salt-losing crisis but rare conditions do occur and must not be overlooked. Often there are genetic 'hotspots' for these conditions due to founder effects in certain populations or other clues to the diagnosis.

Reaching a specific diagnosis can have important implications for the child and its family in terms of treatment, long-term outcome, possible associated features that might need to be considered and counselling the family about recurrence risk. Sometimes, other family members who might be in a pre-symptomatic stage of the disease can be identified and treated before the onset of an adrenal crisis.

The pediatric endocrinologist should play a key role in the diagnostic process, as well as in educating the family and young person as they grow up, establishing long-term treatment plans and putting emergency regimens in place. Although several conditions tend to present at typical ages or with certain features, it is emerging that there is a much greater overlap than previously thought (Figure 9.15). Genetic analysis is playing an increasingly important role in reaching a specific diagnosis.

The term 'Addison disease' has been used broadly in historical descriptions of adrenal disease. Here, we will restrict the use of this term to autoimmune AI and describe other forms as PAI.

Presentation of AI

The presentation of AI varies depending on (1) whether it is primary or secondary, (2) which steroid system is affected (mineralocorticoids, glucocorticoids, androgens), (3) whether it is acute or chronic and (4) associated features or consequences of the condition (Figure 9.16). The physiology of the HPA axis changes with age and can be influenced by general health, coexisting endocrinopathies, stress and fluid/salt intake.

Hyperpigmentation

Hyperpigmentation is a classic feature of PAI and can be useful in differentiating primary disease from secondary (central) or iatrogenic (suppressive) causes. Primary glucocorticoid insufficiency with low cortisol production reduces negative feedback on the hypothalamus and pituitary, resulting in elevated ACTH secretion. As described previously, ACTH is cleaved from the POMC precursor together with other small peptides such as α-MSH. Hyperpigmentation results from increased stimulation of the melanocyte melanocortin 1 receptor (MC1R) by α-MSH as well as possible signalling through this receptor by extremely high concentrations of circulating ACTH.

Hyperpigmentation is most pronounced around the nail beds, knuckles, creases of the palms and gums, in the axilla and in the flexor surfaces (Figure 9.17). Generalized skin pigmentation can occur, which is often most pronounced in children with naturally darker skin. Hyperpigmentation can be present at birth in children with congenital glucocorticoid insufficiency or may show gradual onset during childhood due to progressive adrenal disease. The hyperpigmentation is increased following exposure to the sun and may not be noticed initially. It can be useful to look at serial photographs or to compare the child's pigmentation with that of other family members.

Mineralocorticoid Insufficiency

Mineralocorticoid insufficiency generally results from low aldosterone secretion and typically presents with weight loss, hypotension and collapse. Biochemical evidence of hyponatraemia and hyperkalaemia is present. The most vulnerable time for a child to present with mineralocorticoid insufficiency is in the neonatal period. During late pregnancy, the fetal electrolyte balance is in homeostasis with the mother's so, unless she has renal dysfunction or related conditions, the baby should be born with normal

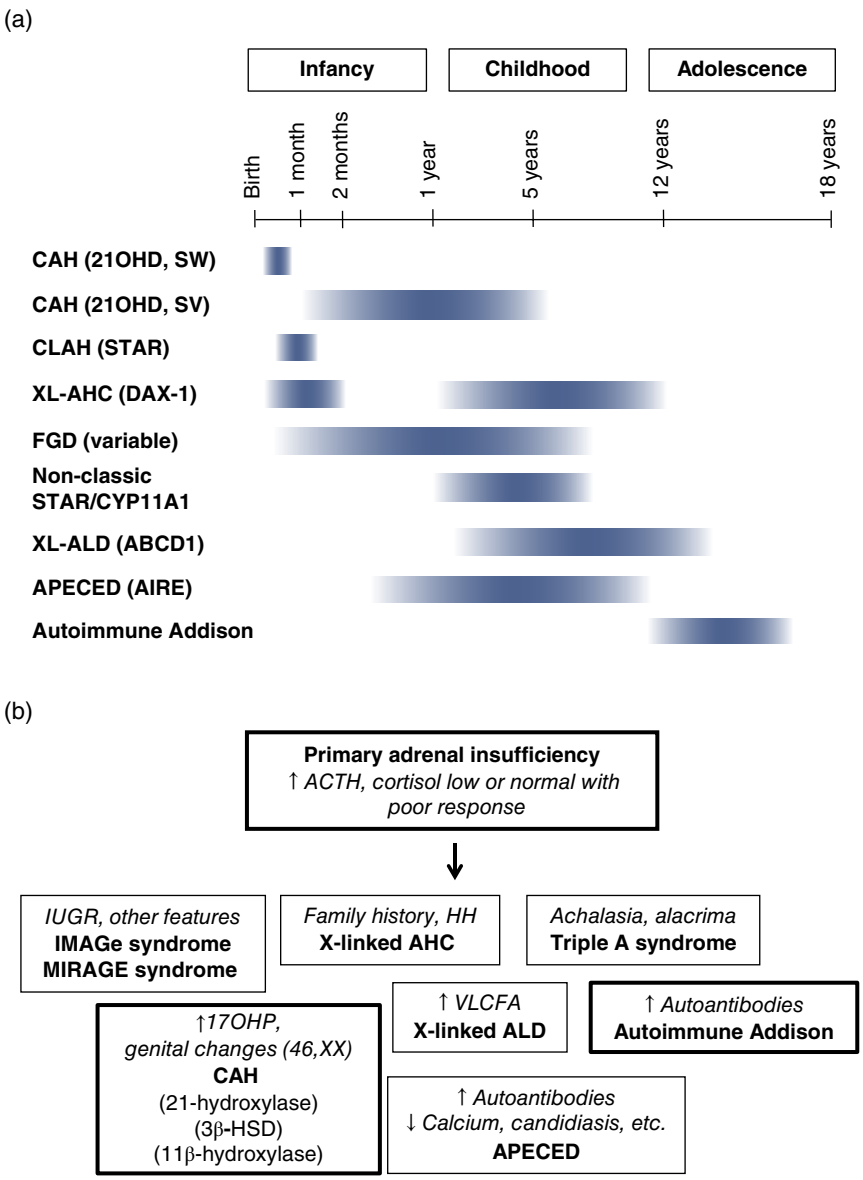


Figure 9.15 (a) Typical ages at presentation of several of the more common or important forms of primary adrenal insufficiency (PAI). Note the non-linear scale. CAH, congenital adrenal hyperplasia; SW, salt wasting; SV, simple virilizing; XL, X-linked; AHC, adrenal hypoplasia congenita; FGD, familial glucocorticoid deficiency; ALD, adrenoleukodystrophy; APECED, autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy/dysplasia. (b) Key diagnostic tests, clinical features or history that can help determine a specific cause of PAI. CAH and autoimmune Addison's disease are the two most common conditions (highlighted). IUGR, intrauterine growth restriction; HH, hypogonadotropic hypogonadism; 17-OHP, 17-hydroxyprogesterone; VLCFAs, very long chain fatty acids.

electrolyte concentrations. The newborn typically produces very high concentrations of aldosterone and renin, as shown by the normal ranges in neonates, which are considerably higher than in children or adults (Figure 9.14). High aldosterone secretion is probably necessary because young infants have relative mineralocorticoid resistance in the kidney, coupled with low sodium intake in breast milk and unpredictable fluid availability when the mother's milk flow is not yet established.

Babies with mineralocorticoid insufficiency start to lose whole body sodium from day 1 in their urine. In the first week of life, increased urinary sodium increases and a progressive elevation in serum potassium concentrations, coupled with progressive hyponatraemia, follows. The whole body sodium and water loss usually results in weight loss (>10% of birth weight), hypotension and circulatory collapse and death if left untreated. Although these changes occur after birth, the biochemical evidence starts to emerge after 4–7 days and children typically present from around 7 days of age.

Aldosterone secretion is normally lower in children and adults than in infants, so aldosterone insufficiency

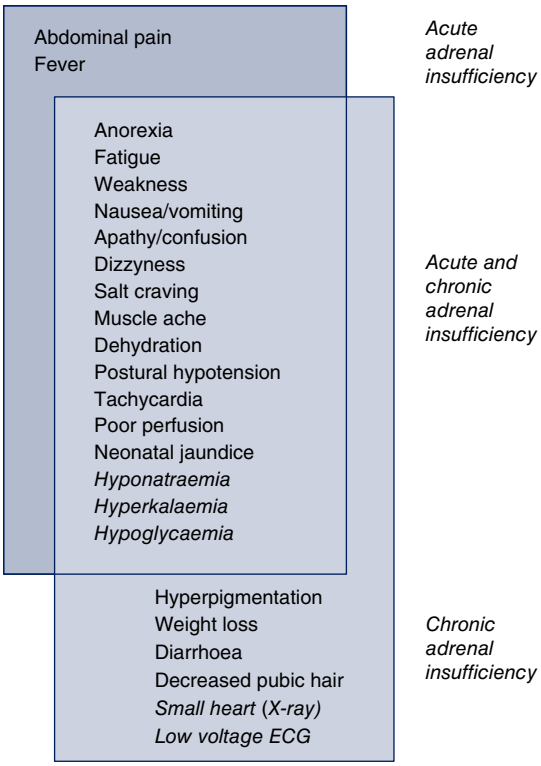


Figure 9.16 Common features of acute and chronic primary adrenal insufficiency.

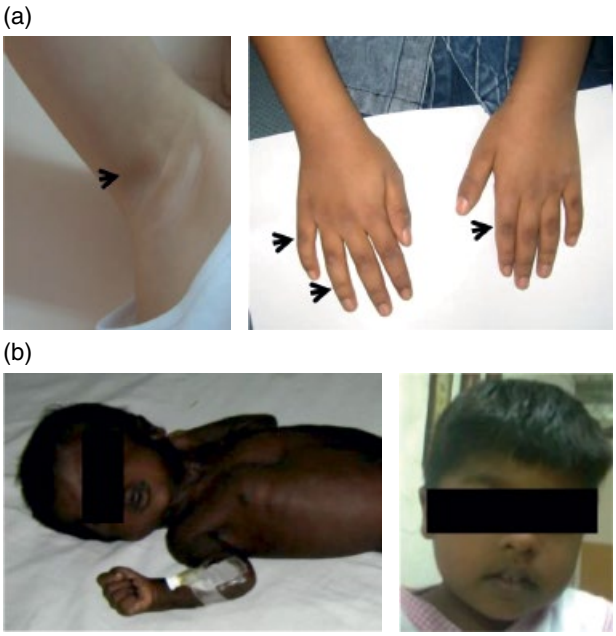


Figure 9.17 Clinical signs of hyperpigmentation associated with primary adrenal insufficiency. (a) Hyperpigmentation of the axilla and knuckles (arrows). Photographs published with consent and courtesy of Professor Lou Metherell. (b) Generalized hyperpigmentation in the newborn period in a baby with ACTH resistance (left panel). The same child is shown after several years of steroid replacement (right panel). (*Source:* Reproduced from Jain et al. [13]. © The Authors under a CC-BY-3.0 licence.) (*See insert for colour representation of the figure*.)

that develops during these times can be subtle. Children can still present with hypotension, often with a postural drop in blood pressure or dizziness on standing, and electrolyte disturbances such as hyponatraemia. Presentation can sometimes be precipitated by low dietary sodium, reduced fluid intake or by stress, illness or a hot climate. There may be a history of excessive salt intake or salt craving, which can delay or mask the presentation of aldosterone insufficiency.

Glucocorticoid Insufficiency

Glucocorticoid insufficiency usually equates with cortisol insufficiency and can present in many ways, in addition to hyperpigmentation (Figure 9.16). In infancy, cortisol is extremely important in maintaining glucose regulation, so hypoglycaemia is a critically important component of AI to monitor, diagnose and treat. Hypoglycaemic convulsions are a frequent presentation of some genetic forms of severe glucocorticoid insufficiency. Hypoglycaemic risk is also higher if there is coexistent growth hormone insufficiency. Cortisol is necessary for bilirubin metabolism and neonates with congenital glucocorticoid insufficiency often have prolonged conjugated hyperbilirubinaemia. Hypocortisolaemia can be associated with poor weight gain, hypotension and respiratory distress syndrome in term babies with apparent hypothyroidism. Some infants with severe glucocorticoid insufficiency and ACTH resistance present with hyponatraemia probably due to a combination of the facilitative role of ACTH on mineralocorticoid synthesis, a supportive role for glucocorticoids on MR function and the fact that cortisol promotes renal free water clearance so that affected hypocortisolaemic children are more likely to get dilutional hyponatraemia.

Glucocorticoid insufficiency can have more subtle presentations in childhood and teenage years such as decreased appetite and weight loss, as well as progressive lethargy and malaise. In the early stages, presentation may be delayed if weight loss and an ability to tan easily are viewed positively. Cognitive function may be affected and ultimately collapse and death can occur. Since many children receive steroids for unrelated conditions, these treatments can delay or mask the onset of genuine primary adrenal disease.

Adrenal Androgen Insufficiency

Dysfunction of the *zona reticularis* and decreased adrenal androgen production can be seen in later childhood as a lack of adrenarche but this is rarely a presenting feature. In females, adrenal androgen insufficiency can be associated with reduced pubic hair or axillary hair at puberty. DHEA has been reported to play a role in cognitive function and libido in women but the magnitude of this effect and benefits of DHEA replacement in women with AI are still unclear.

Associated Features

Infants and children with or at risk of AI sometimes present because of associated features (Table 9.4). For example, children with steroidogenic defects can present with atypical (ambiguous) genitalia at birth. Most commonly this is a 46,XX child with 21-OHD and treatment is needed before the onset of a salt-losing adrenal crisis. Other examples include:

- Boys with X-linked adrenoleukodystrophy (X-ALD) who develop adrenal dysfunction later.
- Children with Triple A syndrome (AAAS) in whom alacrimia and achalasia of the oesophagus can precede the adrenal disorder.
- Short stature associated with defects in SAMD9, CDKN1C, POLE1 or minichromosome maintenance 4 (MCM4).
- Steroid-resistant nephrotic syndrome and sphingosine-1-phosphate lyase 1 (SGPL1).
- Autoimmune conditions such as polyglandular autoimmune endocrine disorders where other features such as hypocalcaemia, fungal infections or alopecia are present (see Chapter 11).

Diagnosing AI and its Causes

In some situations the diagnosis of AI is clear. There is often a need to start treatment urgently but thought should be given to obtaining samples to reach a specific diagnosis since this may not be possible once treatment has started. There is a narrow window of opportunity to obtain relevant samples that can be stored for later analysis. Close liaison with the relevant laboratories is necessary.

It is important to document the clinical status of the child at the time of sampling as this might influence interpretation of the results. For example, a 'normal range' cortisol in a child who is hypotensive and collapsed is *inappropriately low* for the clinical scenario. Similarly, a high potassium concentration in a venous blood sample that has been difficult to obtain from a child who is peripherally shut down may represent haemolysis rather than hyperkalaemia. If this is not noted at the time, subsequent data interpretation can be difficult.

History

A detailed history can be extremely valuable for diagnosing AI and its possible cause. The questions asked depend on the age of the child but can include information related to pigmentation, prolonged jaundice, symptoms of hypoglycaemia, hypotension or dizziness, poor appetite and weight loss, fatigue and poor general health. An adrenal crisis can present with severe weakness, syncope or confusion or with acute abdominal pain, nausea and vomiting and fever (Figure 9.16).

Clues to the underlying cause may come from the age of the child, the presentation, associated features or family history (Figure 9.15). Adrenal disorders are often inherited in a recessive manner (Table 9.4) but several are X-linked (e.g. X-linked AHC due to DAX-1/NR0B1 or X-ALD) and others can be dominant/*de novo* or imprinted. A detailed family history is important, bearing in mind that unexplained deaths of children, 'sudden infant death' or sepsis/gastroenteritis may in fact have been an undiagnosed adrenal condition, especially if detailed endocrine investigation or post-mortem examination was not performed.

Contributing iatrogenic factors should be elucidated including history of recent steroid use but also, for example, inhibition of cortisol synthesis by ketoconazole, aminoglutethimide or etomidate and activation of glucocorticoid metabolism by anticonvulsants like phenytoin or phenobarbital or antibiotics such as rifampicin. These factors are more commonly seen in adults but may be relevant for some children and young people.

Examination

Patients with chronic PAI demonstrate hyperpigmentation, hypotension with increased postural drop, weakness, apathy and fatigue. Children may present with weight loss and failure to thrive. Patients with acute AI and adrenal crisis present with dehydration, hypotension and shock with reduced consciousness and confusion and may have abdominal tenderness and guarding, hyperthermia and hypoglycaemia. If the cause of the AI is acute (e.g. haemorrhage), hyperpigmentation may not have developed. Sometimes an acute crisis can occur on the background of chronic disease.

Deficient adrenal androgen secretion will compromise the development of pubic and axillary hair in adolescent girls.

Additional signs may arise depending on the underlying cause; patients with common forms of CAH demonstrate signs of cortisol deficiency but also of androgen excess (varying degrees of virilization, pubic and axillary hair, acne, hirsutism, increased muscle mass, accelerated growth with a disproportionate increase in bone age and clitoromegaly or penile enlargement) due to accumulated steroidal precursors proximal to the block.

In a boy presenting with inappropriate virilization, it is essential to examine and measure the testes to determine if the androgen excess is likely to be testicular in origin; bilaterally enlarged testes suggest true central precocious puberty, unilateral testicular enlargement suggests testicular tumour and prepubertal-sized testes indicate an extratesticular source of androgen such as the adrenal gland.

Table 9.4 Monogenic causes of primary adrenal insufficiency.

Condition	Gene	Protein	OMIM	Chromosome	Inheritance	Action	Associated features
Adrenal hypoplasia							
X-linked AHC	*NR0B1*	DAX-1	300200	Xp21.2	X-linked	Transcription factor	Hypogonadotropic hypogonadism; impaired spermatogenesis; *early puberty*
Steroidogenic factor 1	*NR5A1*	SF-1	184757	9q33.3	AD, AR, SLD	Transcription factor	46,XY DSD; asplenia
IMAGe syndrome	*CDKN1C*	CDKN1C	614732	11p15.4	Imprinted (maternally expressed)	Cell cycle regulator	IUGR; metaphyseal dysplasia; genital anomalies; *diabetes mellitus*
IMAGEI syndrome	*POLE1*	POLE1	618336	12q24.33	AR	Cell cycle regulator	IMAGE-like, immunodeficiency
MIRAGE syndrome	*SAMD9*	SAMD9	610456	7q21.1	AD (*de novo*)	Endosome function	Infections; IUGR/preterm; gonadal dysfunction; enteropathy; anaemia, thrombocytopaenia; risk of monosomy 7 and myelodysplastic syndrome
SERKAL syndrome	*WNT4*	WNT4	611812	1p36.12	AR	Cell signalling	46,XX DSD; renal dysgenesis; pulmonary hypoplasia
Familial glucocorticoid deficiency (FGD)-like conditions (ACTH resistance)							
FGD1	*MC2R*	ACTH receptor	202200	18p11.21	AR	Cell signalling	Tall stature (pretreatment)
FGD2	*MRAP*	MC2R accessory protein	607398	21q22.11	AR	Cell signalling	—
Non-classic lipoid CAH (steroidogenic acute regulatory protein) (FGD3)	*STAR*	STAR	609197	8q13.2	AR	Steroidogenesis	Possible gonadal dysfunction
Non-classic P450scc insufficiency	*CYP11A1*	P450scc	613743	15q24.1	AR	Steroidogenesis	Possible gonadal dysfunction
Nicotinamide nucleotide transhydrogenase insufficiency	*NNT*	NNT	614736	5p21	AR	Oxidative stress	Early puberty
Thioredoxin reductase 2 insufficiency	*TXNRD2*	TXNRD2	606448	22q11.21	AR	Oxidative stress	Heart defects
Minichromosome maintenance 4	*MCM4*	MCM4	609981	8q11.21	AR	DNA repair	Natural killer cell defects, microcephaly, postnatal growth failure
Triple A syndrome (Allgrove syndrome)	*AAAS*	ALADIN	23150	12q13.13	AR	Nuclear pores, oxidative stress	Achalasia, alacrimia, ataxia/ neurological involvement, hyperkeratosis
Sphingosine-1-phosphate lyase 1 insufficiency	*SGPL1*	SGPL1	603729	10q22.1	AR	Sphingolipid metabolism	Steroid-resistant nephrotic syndrome, ichthyosis, neurological involvement

(*Continued*)

Table 9.4 (Continued)

Condition	Gene	Protein	OMIM	Chromosome	Inheritance	Action	Associated features
Autoimmune adrenalitis							
Autoimmune polyglandular syndrome type 1 (APS-1) (APECED)	*AIRE*	Autoimmune regulator	240300	21q22.3	AD, AR	Immune regulation	Hypoparathyroidism, mucocutaneous candidiasis, alopecia, pernicious anaemia, other autoimmune features
Metabolic causes							
Smith–Lemli–Opitz syndrome	*DHCR7*	7-Dehydrocholesterol reductase	270400	11q13.4	AR	Cholesterol metabolism	Syndactyly, facial features, microcephaly, cardiac defects, hypospadias
X-linked adrenoleukodystrophy	*ABCD1*	ABCD1	300100	Xq28	X-linked	Membrane transporter	Neurological dysfunction
Neonatal adrenoleukodystrophy	*PEX genes; related genes*	Peroxins	Several	Several	AR	Peroxisome biogenesis	Neurological, facial features, hepatic dysfunction
Primary xanthomatosis (Wolman disease); cholesterol ester storage disease	*LIPA*	Cholesterol ester	278000	10q23.31	AR	Cholesterol metabolism	Failure to thrive, steatorrhoea, hepatosplenomegaly adrenal calcification
Mitochondrial disorders (Kearns–Sayre syndrome; Pearson syndrome; others)	*Mitochondrial DNA; MRPS7; NDUFAF5; GFER*	Several	Several	Several	Maternally inherited or AR	Mitochondrial function	Variable multisystem features
Glucocorticoid resistance							
Glucocorticoid resistance (Chrousos syndrome)	*NR3C1*	Glucocorticoid receptor (GRα)	615962	5q31.3	AD, AR	Transcription factor	Fatigue, hypertension, hirsutism, obesity
Disorders of aldosterone synthesis and action							
Aldosterone synthase insufficiency	*CYP11B2*	P450c11AS	203400 610600	8q24.3	AR	Steroidogenic enzyme	—
Mineralocorticoid resistance (pseudohypoaldosteronism type 1)	*NR3C2*	Mineralocorticoid receptor (MR)	177735	4q31.23	AD	Transcription factor	—
Congenital adrenal hyperplasia							
Congenital lipoid adrenal hyperplasia	*STAR*	STAR	201710	8p11.23	AR	Steroidogenic enzyme	46,XY DSD; impaired gonadal steroidogenesis
P450 side-chain cleavage insufficiency	*CYP11A1*	P450scc	613743	15q24.1	AR	Steroidogenic enzyme	46,XY DSD; impaired gonadal steroidogenesis

3β-Hydroxysteroid dehydrogenase type II insufficiency	*HSD3B2*	3β-HSD2	201810	1p12	AR	Steroidogenic enzyme	46,XY DSD; impaired gonadal steroidogenesis; 46,XX clitoromegaly
17α-Hydroxylase/17,20-lyase insufficiency	*CYP17A1*	P450c17	202110	10q24.32	AR	Steroidogenic enzyme	46,XY DSD; impaired gonadal steroidogenesis; hypertension
P450 oxidoreductase	*POR*	P450 oxidoreductase	201750	7q11.23	AR	Steroidogenic enzyme	Antley–Bixler syndrome (craniosynostosis, skeletal features, choanal atresia); atypical genitalia (46,XY and 46,XX); impaired gonadal steroidogenesis at puberty
21-Hydroxylase deficiency	*CYP21A2*	P450c21	201910	6p21.33	AR	Steroidogenic enzyme	46,XX DSD; virilization, early puberty
11β-Hydroxylase deficiency	*CYP11B1*	P450c11	202010	8q24.3	AR	Steroidogenic enzyme	46,XX DSD; virilization, early puberty, hypertension

Source: Modified from Achermann [1].

Associated features can also provide important diagnostic clues (discussed above; Table 9.4).

General Investigations for AI

The diagnosis of AI is characterized by a low morning plasma or serum cortisol concentration or an inappropriately low or normal cortisol concentration during a time of stress and is confirmed by a low stimulated cortisol concentration following dynamic testing. Circadian rhythms may not be well established in children younger than 3 years of age. An elevated basal ACTH is useful to support the diagnosis of PAI but will not be elevated in secondary AI.

Morning Cortisol Concentrations

There is debate regarding the morning cortisol concentration that suggests inadequate adrenal function. A large meta-analysis found the diagnosis of PAI most likely if the cortisol were <140 nmol/L in combination with an ACTH concentration > twofold above the upper limit of normal for the specific assay. There is also controversy determining what cortisol concentration rules out AI with studies stating concentrations ranging from 285 to 480 nmol/L. Many children with significant adrenal compromise have a mid-range cortisol and elevated ACTH but a poor response to further stimulation. Therefore, isolated 'normal' cortisol values (e.g. 200–350 nmol/L) should be taken with caution, especially in the sick child when cortisol should be higher.

Stimulation Tests: Insulin Tolerance Test and Synacthen Test

The diagnosis of AI should always be confirmed by a stimulation test if possible. Various dynamic tests have been devised to assess whether the patient can produce a response like the normal stress-induced rise in a healthy person with a normal HPA axis.

To define the normal stress response in healthy patients, the peak serum cortisol response was assessed in a large cohort of patients undergoing major surgery. The lowest concentration recorded was taken as the threshold for diagnosing AI during the insulin tolerance test (ITT). This test uses a small bolus of insulin to induce symptomatic hypoglycaemia as the 'stress' stimulus. Its advantages include the application of a known stimulus and a predictable and defined response within 30 minutes to that stimulus, namely, hypoglycaemia (<2.6 mmol/L on lab glucose but ≤3 mmol/L on BM stix) or a glucose decrement from baseline of > 50%. It tests the integrity of the entire HPA axis and has the benefit of also testing growth hormone reserve as the hypoglycaemia reverses itself in response to counter-regulatory hormones (ACTH and cortisol, growth hormone, epinephrine and glucagon). The ITT is potentially dangerous and requires a high degree of supervision from experienced and trained clinicians and is not appropriate for young children, especially those <2 years of age or those with a history of epilepsy. Special precautions are required in children with suspected panhypopituitarism or those who have received cranial irradiation.

The corticotropin stimulation test, also known as the short Synacthen test, is currently regarded as the gold standard diagnostic test for PAI as it has been validated against the ITT for diagnostic accuracy. It is a rapid, safe and easy test to evaluate adrenocortical function. The original ACTH test consisted of a 4–6 hours infusion of 0.5 units/kg of ACTH (1–39) to stimulate adrenal cortisol secretion maximally. This has been replaced by the standard short Synacthen test using synthetic ACTH (1–24) (cosyntropin or tetracosactrin [Synacthen]), which has a more rapid action and shorter half-life than ACTH (1–39).

The Synacthen test is used with different protocols regarding route of administration, dose of Synacthen used and duration of test. Commonly the test involves collecting a basal sample cortisol at 0 minutes and stimulated concentrations at 30 and 60 minutes after intravenous (IV) (or intramuscular [IM]) administration of a bolus of 250 μg Synacthen. The standard Synacthen test is not significantly affected by diurnal variations and can be carried out at any time of the day. The 30 minute time point has been validated against the ITT but concentrations rise further at 60 minutes. A large meta-analysis showed significant variability across studies in optimal timing for measuring cortisol response, but showed that in no study was there a statistically significant difference in diagnostic discrimination at 30 or 60 minutes [14–16].

The Endocrine Society has recently published a clinical practice guideline on the diagnosis and treatment of PAI. This document provides clear guidelines for both adults and children regarding whom to investigate and optimal treatment regimens. The guideline recommends the standard dose of 250 μg corticotropin test for adults and children >2 years of age, 125 μg for children <2 years and 15 μg/kg for infants. The preferred route is as an IV bolus with cortisol response measured at 30 or 60 minutes. Peak cortisol concentrations below 500 nmol/L (18 μg/dL) at 30 or 60 minutes indicate AI, noting that different laboratories will have different assays using different detecting antibodies and therefore slightly different normal values [17].

In acutely unwell patients, treatment should not be delayed until test results are available but a single baseline sample to measure ACTH and cortisol before administration of hydrocortisone can provide invaluable information and be essential for retrospective confirmation of the diagnosis. Serum/plasma should be stored and additional tests discussed with the laboratories once the clinical picture becomes more established.

ACTH Measurement

The diagnosis of PAI can be confirmed by measuring ACTH. A low morning plasma or serum cortisol concentration in combination with an elevated plasma ACTH concentration > twofold the upper limit of the reference range is consistent with PAI. An ACTH concentration >300 ng/L (66 pmol/L) represents a maximal stimulus for cortisol secretion and if found in combination with a low or even relatively normal cortisol concentration suggests that the adrenal cortex is unable to respond to ACTH.

Electrolytes, Plasma Renin Activity and Aldosterone

In addition to measuring plasma cortisol and ACTH to establish PAI, it is important to measure electrolytes, PRA and aldosterone to determine the presence or absence of mineralocorticoid deficiency simultaneously. The normal ranges of PRA and aldosterone are much higher in infancy and early childhood and this needs to be considered when interpreting results, since some laboratories may quote normal ranges for adults (Figure 9.14). Urinary sodium can sometimes be used to monitor for renal salt loss.

Diagnosing the Specific Cause of AI

It should be the aim always to try to determine the aetiology of PAI in patients with confirmed disease. Algorithms used in adult practice cannot be applied to children: the commonest cause of AI in infancy is CAH due to 21-hydroxylase insufficiency in contrast to adults who most commonly have autoimmune adrenalitis (Addison disease). There is rarely a need for a CT scan of the adrenal glands in children with AI but additional genetic investigations are often required to establish the diagnosis of a specific syndrome, rare form of CAH or familial glucocorticoid deficiency (FGD) or AHC. An overview of the most common causes of AI in children, typical ages at presentation and useful diagnostic features is shown in Figures 9.15 and 9.16.

17-Hydroxyprogesterone (17-OHP) and Intermediary Steroids

A baseline 17-OH progesterone concentration can be diagnostic for 21-OHD, especially if concentrations are >300 nmol/L (1000 ng/dL). However, it is established practice to confirm the diagnosis of CAH using the standard Synacthen test if possible, investigating both cortisol and 17-OH progesterone concentrations at baseline and 30 (±60) minutes. Stimulating the adrenal gland with ACTH increases steroidogenesis, resulting in the further accumulation of steroids proximal to the enzyme block (Figure 9.6; see also Figure 9.20). Comparing the patient's basal and ACTH-stimulated values of 17-OHP against those from large numbers of well-studied patients usually allows the discrimination of normal individuals, heterozygotes, patients with non-classic CAH and patients with classic CAH, although there is inevitably some overlap between groups (see *21-OHD* and Figure 9.23). Measurement of testosterone or androstenedione in response to ACTH can distinguish unaffected people from patients with classic CAH but heterozygotes and patients with cryptic CAH have values overlapping both normal and classic CAH.

17-OHP may be elevated in enzymatic defects other than 21-OHD, which include 11β-hydroxylase deficiency, PORD and 3β-HSD2 deficiency. The concentrations of 17-OHP measured in these conditions are usually less than in 21-OHD. Samples from preterm babies and samples taken within the first 24–48 hours of life have higher 17-OHP using conventional assays. Plasma 17-OHP can be raised in babies with stress (e.g. cardiac) and in children with some adrenal and testicular tumours. Analysing additional intermediary steroids such as DOC, 11-deoxycortisol, 17-OH pregnenolone, DHEA and androstenedione before and after stimulation can sometimes help to differentiate various enzymatic defects.

Many countries have now incorporated screening for CAH due to 21-OHD into their newborn screening programmes. This is an attractive disease to screen for as it is relatively common (1 : 13,000–1 : 15,000) and can be fatal but early diagnosis and treatment can prevent significant morbidity and mortality. Immunoassays are usually used to measure 17-OHP in dried blood spots on filter paper (Guthrie cards) as part of the screening protocol. Approximately 1% of all tests are reported as positive to obtain adequate sensitivity but only ~1% of these babies with positive tests have CAH. Guidelines suggest that a second-tier approach could improve the positive predictive value of screening; suggested methods include using liquid chromatography followed by tandem mass spectrometry (LC-MS/MS) or genotyping samples for one of the 10 most common mutations that cause CAH.

All infants with positive newborn screening for CAH need to be investigated urgently to confirm or exclude the diagnosis and to start treatment before a salt-losing crisis. This can be a major challenge in some countries because of geographical aspects or when the established newborn screening programme is carried out after several days. As the assay methods and protocols vary in different regions, it is important that pediatric endocrinologists are familiar with local cut-offs and pathways. Since more than 70% of 46,XX girls with CAH have atypical genitalia at birth, medical professionals should be alerted to the possible diagnosis; the main advantage of screening is to diagnose boys with CAH.

Urine Steroid Analysis

Urine steroid analysis is a useful tool that can be used both quantitatively and qualitatively. The most common quantitative analysis is of total UFC. The amount of cortisol secreted varies depending on age, size and several other factors. UFC has a useful role sometimes in assessing excess cortisol production (see Cushing Syndrome) but is rarely used for the assessment of AI. The UFC is typically performed from a complete 24 hour urine collection.

The urine steroid profile (USP) is a more detailed analysis of both the quantity and characteristics of steroid metabolites in the urine. This test is best done following a 24 hour collection but an indication of steroid patterns and relative amounts can be performed on a 'spot' urine sample of just a few millilitres.

The USP is performed by gas chromatography–mass spectrometry (GC-MS). The gas chromatographic component separates the different steroids in the urine, most of which are glucuronidated or sulphated (in early infancy). The mass spectrometry analyses the molecular weight of each major fraction and daughter derivatives to identify the specific steroid.

USP is available mostly in specialist laboratories but is extremely useful for supporting a diagnosis of many adrenal steroidogenic defects (e.g. 21-OHD, 11β-hydroxylase deficiency, PORD, 3β-HSD2 deficiency, 17α-hydroxylase deficiency) and disorders of mineralocorticoid function (e.g. aldosterone synthase deficiency, apparent CRD). The USP can also reliably diagnose 5α-reductase deficiency type II but only after 4–6 months of age.

Adrenal Autoantibodies

Children older than 6 months should have screening for autoimmune adrenalitis with P450c21 autoantibodies but laboratory testing for autoantibodies is not standardized and is subject to wide between-method variation. In children with confirmed autoimmune PAI, autoimmune polyglandular syndrome type 1 (also called APS-1 or autoimmune polyendocrinopathy-candidiasis–ectodermal dystrophy/dysplasia [APECED]) and autoimmune polyglandular syndrome type 2 (APS-2) should be considered (see Chapter 11). APS 1 is most frequently associated with chronic mucocutaneous candidiasis and hypoparathyroidism and can be screened for with measurement of antibodies to interferon ω or α that have a high diagnostic sensitivity and specificity. APS type 2 is associated with autoimmune thyroid disease and type 1 diabetes. In addition to autoantibody screens, relevant biochemical testing (e.g. calcium, PTH, thyroid function) should be considered.

Very Long Chain Fatty Acids (VLCFAs)

All preadolescent boys and older males without an established diagnosis should be screened for adrenoleukodystrophy (ALD) by measuring very long chain fatty acids (VLCFAs) (C26 : 0 and #c26 : 0/C22 : 0 and #c24 : 0/C22 : 0 ratios). This condition most often presents in boys aged 2–10 years and AI can be the presenting sign in a subset of patients. Rare neonatal forms of ALD can also occur.

Imaging

Imaging of the adrenal gland can involve ultrasound, X-ray, MRI, CT or more specialized tracer uptake scans (e.g. MIBG) used for rare tumours (e.g. neuroblastoma) or phaeochromocytoma. In general, adrenal imaging has limited use in the diagnosis of many adrenal conditions in children. For example, CAH can cause enlargement of the adrenal glands, especially in CLAH (*STAR*), but many children have normal adrenals on scans. Conversely, small or absent adrenal glands are not always clearly documented in children with adrenal hypoplasia. Imaging can be important to detect physical causes of AI such as haemorrhage or infiltration (e.g. tumour) and adrenal calcification is seen in tuberculosis as well as in some rare genetic conditions (e.g. SGPL1 deficiency, Wolman disease). Imaging can also be useful in the diagnosis of adrenal tumours and in the investigation of CS. Adrenal incidentalomas are less common in children than in adults, where they can complicate interpretation but non-specific lesions of unknown significance do occur and experienced radiological review is important.

Karyotype

All children with hypospadias or atypical genitalia should have a karyotype or array performed for sex chromosomes. Severe forms of CAH (e.g. 21-hydroxylase, 11β-hydroxylase) should be considered in a baby with impalpable testes. Phenotypic females presenting in early infancy with salt-losing AI should have a karyotype to ensure that a 46,XY child with a high steroidogenic block (e.g. *STAR*, *CYP11A1*) is not missed.

Genetic Testing

Children, especially neonates and infants, presenting with PAI and low 17-OH progesterone concentrations require further investigation determined by family history, presence or absence of mineralocorticoid deficiency and additional associated features. Further investigations may include additional steroid precursors and urine steroid profiling but early genetic analysis is becoming more established.

Genetic testing can be focused on a single gene when there are associated features that suggest a diagnosis, when there is a family history (e.g. of X-linked disease) or when the importance of making a specific diagnosis is high (e.g. NR0B1/DAX-1). Many forms of PAI do not have specific features so next-generation sequencing

approaches to the analysis of many genes at once are becoming increasingly used, in clinical practice as well as on a research basis.

The most cost-effective approach currently is to use targeted panels of multiple genes known to cause PAI, sequencing many relevant genes at once. A recent study reported analysis of 95 children with previously undiagnosed PAI in Turkey, where CAH, metabolic and syndromic causes of PAI had been largely excluded. Targeted panels were used to reach a diagnosis in 82% of them [18]. The diagnoses involved just nine key known genes (*MC2R*, DAX-1/*NR0B1*, non-classic *STAR*, non-classic *CYP11A1*, *MRAP*, *NNT*, SF-1/*NR5A1*, X-ALD/*ABCD1* and *AAAS*). Although this high diagnostic yield reflects the high degree of consanguinity to some extent, the diagnosis rate of children with isolated PAI is now more than 50% in populations where consanguinity is low.

Alternative approaches using next-generation sequencing strategies include whole exome and whole genome sequencing. These technologies are currently more expensive and research based, but will become increasingly available as diagnostic clinical tests once costs drop and as bioinformatic analysis becomes more streamlined. Next-generation sequencing approaches have been used recently to identify novel causes of PAI, such as *SGPL1* and *SAMD9* mutations [19, 20].

Reaching a specific genetic diagnosis can have important implications in terms of personalizing management (e.g. the need for mineralocorticoid replacement), the need to investigate for any associated features (e.g. monitoring gonadal function), predicting long-term outcome and course of the condition, identifying pre-symptomatic family members before the onset of an adrenal crisis and counselling the family regarding the chances of recurrence in future pregnancies. In the future, genetic testing may become a first-line investigation once the diagnosis of AI is established.

Secondary AI

The reference test for establishing the integrity of the HPA axis and therefore diagnosing ACTH deficiency and secondary AI is the ITT. Alternatively, the overnight metyrapone test assesses the response to an interruption of the negative feedback from cortisol. Both tests have drawbacks and so simpler, less invasive and safer alternatives have been investigated including both the standard and low-dose Synacthen tests.

The rationale for the Synacthen test in this context relies on the fact that chronic endogenous ACTH deficiency leads to cortisol deficiency and reduced acute responsiveness of the *zona fasciculata* following stimulation. This test should therefore not be used following an acute insult likely to cause secondary AI, for example, in the immediate post-operative period following pituitary surgery.

The low-dose Synacthen test uses a dose of 1 µg (or 500 ng/m^2) rather than 250 µg. Cortisol is usually measured at baseline and at frequent intervals up to 45–60 minutes. This test provides a higher sensitivity in the diagnosis of secondary AI, including glucocorticoid-induced hypothalamic–pituitary AI, but not PAI, compared with the high-dose test. In a meta-analysis of 12 studies, a basal cortisol <138 nmol/L best predicted secondary AI and values >365 nmol/best predicted normal HPA axis function. Given difficulties in preparing the solution accurately, some authors continue to use the standard short Synacthen test.

Additional Technical Information and Tests

Steroid Measurements

Plasma cortisol can be measured by a variety of techniques including radioimmunoassay and immunoradiometric assay and LC-MS/MS. It is important to know what procedure one's laboratory is using and what is measured, because laboratories have different normal values and most central hospital and commercial laboratories are designed primarily to serve adult, rather than pediatric, patients.

Serum cortisol is usually measured by immunoradiometric assay. Increasingly, high-throughput platforms are used to generate rapid turnaround but, even in adults, cortisol values may vary by up to 20–30% on different bioanalytical platforms. Furthermore, immunoassays have some degree of cross-reactivity with other steroids and most cortisol immunoassays detect cortisol and cortisone, which are readily distinguished by LC-MS/MS. As plasma from newborns contains cortisone rather than cortisol, comparison of newborn data obtained by HPLC with published standards obtained by immunoassays may incorrectly suggest AI.

Total cortisol is measured; for free cortisol values, knowledge of corticosteroid-binding globulin (CBG) concentrations is required. Plasma cortisol is 80% bound to CBG and 10–15% bound to albumin. Patients with disorders that reduce CBG levels, such as inflammation (often seen early post-operative or in intensive care), nephrotic syndrome or liver disease, appear to have lower cortisol measurements. In addition, test results from patients with rare conditions such as cortisol-binding globulin deficiency, glucocorticoid resistance and hypersensitivity will be difficult to interpret.

Patients with increased CBG concentrations will have falsely high cortisol concentrations. Oestrogens affect CBG, so total cortisol concentrations may appear higher in females during puberty, pregnancy and in those receiving hormone replacement with an oral contraceptive pill or oral oestrogens to induce puberty. Transdermal oestrogen does not seem to affect CBG concentrations.

Approximately 5% of the hormone is free and biologically active. This is excreted in the urine and saliva, both of which can be diagnostically useful.

Plasma Renin

Renin is not generally measured directly but assayed by its enzymatic activity. PRA is an immunoassay of the amount of angiotensin I generated per millilitre of serum per hour at 37 °C. In normal serum, the concentration of renin and angiotensinogen (the renin substrate) is limiting. Therefore, another test, plasma renin content (PRC), measures the amount of angiotensin I generated in 1 hour at 37 °C in the presence of excess concentrations of angiotensinogen. Both immunoradiometric and more recently automated chemiluminescent immunoassays (CLIA) are also available for measuring direct renin concentration.

Specimens for renin analysis should be frozen immediately, stored frozen, rapidly thawed and rapidly analysed to avoid either activation of prorenin and increases in PRA and direct renin concentration or spurious reductions in PRA due to renin conversion of angiotensinogen substrate.

LC-MS/MS assays for measuring PRA are becoming more available and have many analytical advantages including wider dynamic ranges and improved analytical specificity but PRA assays are still not standardized internationally.

PRA is sensitive to dietary sodium intake, posture, diuretic therapy, activity and sex steroids. Because PRA values can vary widely, it is best to measure renin twice, once in the morning after overnight supine posture and then again after maintenance of upright posture for 4 hours. A simultaneous 24 hours urine for total sodium excretion is useful to interpret PRA results. Decreased dietary and urinary sodium, decreased intravascular volume, diuretics and oestrogens will increase PRA. Sodium loading, hyperaldosteronaemia and increased intravascular volume decrease PRA.

Renin measurements are commonly used in the evaluation of hypertension and in the management of CAH but several additional situations require assessment of the renin–angiotensin system. Children with 'simple virilizing' adrenal hyperplasia who do not have clinical evidence of urinary salt wasting (hyponatraemia, hyperkalaemia, acidosis, hypotension, shock) may nevertheless have increased PRA, especially when dietary sodium is restricted. This was early evidence that this form of 21-OHD was simply a milder form of the more common severe salt-wasting form. Treatment of simple virilizing 21-OHD with mineralocorticoid sufficient to suppress PRA into the normal range can reduce the child's requirement for glucocorticoids, thus maximizing final adult height. Children with CAH need to have their mineralocorticoid replacement therapy monitored routinely by measuring PRA.

Measurement of angiotensin II is also possible in some research laboratories but most antibodies to angiotensin II cross-react strongly with angiotensin I. Thus, PRA remains the most useful way of evaluating the renin–angiotensin–aldosterone system.

Plasma Aldosterone

Aldosterone is a difficult analyte to measure as there are numerous compounds that can interfere with analysis. Previously aldosterone was measured in serum by radioimmunoassay following a chromatographic 'clean-up'. This approach allowed separation of potentially cross-reacting compounds but was time consuming. More recently, automated CLIA have allowed increased throughput and have improved analytical variability but cannot mitigate the problems with antibody cross-reactivity. Developments in LC-MS/MS have allowed aldosterone to be quantified in a consistently accurate manner but this is expensive and standards can be challenging, so many laboratories continue to use automated immunoassays.

Plasma ACTH

Accurate immunoassay of plasma ACTH is available in most centres but its measurement remains more difficult and variable than the assays for most other pituitary hormones. Some assays of ACTH require samples to be drawn into a plastic syringe containing heparin or ethylenediaminetetraacetic acid (EDTA) and transported quickly in plastic tubes on ice, as ACTH adheres to glass and is quickly inactivated. Other more recent assays are more robust but still sensitive to transport delays and temperature. Therefore, elevated plasma ACTH concentrations can be informative but most assays cannot detect low or low-normal values and such values can be spurious if the samples are handled inappropriately. In adults and older children with well-established circadian rhythms of ACTH, normal 0800 hours values rarely exceed 50 pg/mL, whereas 2000 hours values are usually undetectable. Patients with Cushing disease often have normal morning values but consistently elevated afternoon and evening concentrations can suggest the diagnosis. Patients with the ectopic ACTH syndrome have values from 100 to 1000 pg/mL and ACTH can be extremely high in PAI.

Secretory Rates and Dynamics

The secretory rates of cortisol and aldosterone (or other steroids) were historically measured by administering a small dose of tritiated cortisol or aldosterone and measuring the specific activity of one or more known metabolites in a 24 hour urine collection or in blood. This

procedure is not used in clinical practice but can provide information about the normal rate of production of various steroids. Based on this procedure, a daily cortisol secretory rate of 6–9 mg/m^2 in children and adults was estimated. More recently, stable isotope (deuterated) glucocorticoid tracers have been used to calculate the activity of different enzymes or isoenzymes in human steroid metabolism.

Prolonged Synacthen Test

Longer ACTH tests of up to 3 days have been used to evaluate adrenal function but ACTH has both acute and chronic effects. Thus, short tests measure only the acute effects of ACTH and reflect the maximal stimulation of pre-existing steroidogenic machinery. A 3-day test will examine the more chronic effects of ACTH to stimulate increased capacity for steroidogenesis by increasing the synthesis of steroidogenic machinery. Few situations exist in which a 3-day IM ACTH test is indicated.

CRH Testing

CRH is now generally available as a test of pituitary ACTH reserve. It remains experimental in adults and little experience has been gained from children. Some data suggest that it may be useful for distinguishing hypothalamic from pituitary causes of ACTH deficiency and may also be of some use in establishing the diagnosis of Cushing disease.

Treatment of AI

General Approaches

Treatments for AI would ideally mimic physiological patterns of glucocorticoid and mineralocorticoid secretion but currently available cortisol therapies do not allow physiological replacement and therefore treatment regimens aim to control the symptoms of AI and minimize risk of adrenal crisis while avoiding side effects of overtreatment and, most importantly, permitting normal growth and development.

There are no published randomized controlled trials investigating different cortisol replacement therapies in children. Hydrocortisone is the preferred formulation in children as it has a short half-life, is easier to titrate and has the least growth suppressive effects. The more potent synthetic longer-acting glucocorticoids such as prednisolone and dexamethasone are not recommended in children due to their increased adverse side effect profile (Table 9.2).

Treatment regimens vary depending on the specific form of AI but most suggest hydrocortisone in three or four divided doses with a starting dose of 8 mg/m^2/day ('replacement' doses) increasing to 10–15 mg/m^2/day in conditions such as CAH ('suppression' doses).

Mineralocorticoid replacement is with fludrocortisone; a typical daily dose is 100 µg, ranging from 50 to 200 µg (~100 µg/m^2/day). Infants usually require sodium chloride supplementation of 1–2 g/day (17–34 mmol/day, up to 10 mmol/kg/day) due to relative mineralocorticoid resistance in the immature kidney and the low sodium content of breast and formula milk. Infants should be assessed at least every 3–4 months for signs or symptoms of under- or overtreatment including growth, blood pressure and general well-being. Consistently timed hormone measurements (17-OHP, plasma renin) are useful for monitoring treatment and should indicate the need for dose adjustment before clinical parameters in CAH [21].

Initial Treatment and Emergency Regimen

In all patients presenting with possible AI or adrenal crisis, it is essential to obtain some initial investigations including:

- Blood glucose using a glucometer with confirmatory serum glucose.
- Serum electrolytes to include sodium, potassium and urea.
- Blood gas for acid–base analysis.

If this is the initial presentation in a previously undiagnosed child, the following investigations should ideally be collected before treatment commences but, if this is not possible (e.g. if the patient is too unwell to allow adequate blood sampling), urgent management should commence and samples be taken as soon as possible noting the time between initial treatment and investigations:

- 2 mL serum sample (clotted blood) for cortisol and 17-OH progesterone.
- 2 mL EDTA sample for ACTH, aldosterone and renin concentrations – sent to lab urgently (preferably on ice).
- First available urine sample (5–10 mL) for urinary steroid profile and urinary sodium.

Pediatric patients with a suspected adrenal crisis should receive immediate parenteral hydrocortisone 50 mg/m^2 or 2 mg/kg together with appropriate fluid resuscitation in the form of IV normal saline and ongoing parenteral hydrocortisone 50–100 mg/m^2/day either by IV infusion or 4–6 hourly bolus injections (1–2 mg/kg/dose). The initial stress dose can be estimated as 25 mg for infants and preschool children, 50 mg for school-age children and 100 mg for adolescents and adults. Hypoglycaemia should be treated with IV fluids containing dextrose as per protocol and monitored regularly. Hyperkalaemia will usually respond rapidly to hydrocortisone but in the event of an arrhythmia, the hyperkalaemia can be treated using 10% calcium

gluconate, nebulized salbutamol, sodium bicarbonate, calcium resonium or a glucose and insulin infusion.

Ongoing frequent investigations including blood glucose, electrolytes and blood gas are required to ensure correct treatment, aid appropriate fluid resuscitation and avoid too rapid normalization of hyponatraemia, which can be dangerous. When the child is stable, the IV dose can be tapered and switched to an oral regimen when tolerated. Initially this is usually triple maintenance dose.

In patients with mineralocorticoid deficiency, 9α-fludrocortisone should be started at maintenance doses (usually 50–100 μg daily) as soon as the patient can tolerate oral medications. There is no specific IV mineralocorticoid replacement therapy; initial fluid resuscitation with stress doses of hydrocortisone adequately covers mineralocorticoid replacement therapy, as 20 mg *IV* hydrocortisone is equivalent to ~0.1 mg (100 μg) fludrocortisone. It is important to remember that prednisolone and especially dexamethasone have little to no mineralocorticoid activity and the mineralocorticoid activity of oral hydrocortisone is much lower than that of IV (Table 9.2).

Stress dosing (double or triple maintenance dose) is recommended for patients with febrile illness (>38.5 °C), gastroenteritis with dehydration, surgery accompanied by general anaesthesia and major trauma. Minor illness, exercise and physiological stresses (e.g. school examinations) do not generally require increased glucocorticoid dosing, although this needs to be reviewed for each patient.

The highest cause of mortality in patients with known AI is severe gastroenteritis usually due to a combination of vomiting or poor absorption of steroids, together with hypovolaemia due to decreased fluid intake, vomiting, diarrhoea and loss of fluid into the gut. Therefore, gastroenteritis needs to be taken especially seriously in patients with AI. More details about 'sick day rules' and emergency treatment are outlined in the section on 'education and information for families' [22]. In vulnerable younger children, an extra hydrocortisone dose to be given at 0400 hours, which is equivalent to a doubled early morning dose, is recommended during acute illnesses.

If a child with suspected undiagnosed AI dies, attempts should be made to store serum and urine for hormone analysis and blood or tissue for DNA. Post-mortem examination should look specifically for adrenal weight and histology and any associated features. Establishing a diagnosis in such situations can have important implications for the family and for future pregnancies.

Glucocorticoid Replacement

Glucocorticoid replacement therapy is a delicate balance of attempting to prevent the undesirable side effects of both under- and overtreatment. Undertreatment may impair patients' capacity to respond to stress and cause symptoms consistent with AI. Overtreatment can cause the signs and symptoms of Cushing syndrome (CS) and even minimal overtreatment can impair growth.

Estimates of normal endogenous cortisol secretion rates can be used to optimize replacement therapy and have been estimated to be about 5–8 mg/m^2/day, which equates to ~15–25 mg/day *oral* hydrocortisone in adults. Mean cortisol production rates are influenced by age and body composition and vary considerably even among children of the same size compounding the difficulties in tailoring a specific child's replacement regimen. In addition, although glucocorticoids are secreted in a circadian rhythm with a peak in the morning and low concentrations in the evening, there is also a pulsatile pattern with cortisol released episodically during the day in response to various physiological demands. A planned replacement regimen cannot anticipate these day-to-day variations.

Treatment regimens differ depending on the aetiology of AI. In patients with, for example, autoimmune adrenalitis, guidelines suggest starting at hydrocortisone doses of 8 mg/m^2/day in three to four divided doses with doses adjusted according to individual need, usually the largest dose on waking with a second dose after lunch and the third a few hours before bed but in patients with CAH, undertreatment carries the additional risk of hyperandrogenism, virilization and an increase in bone age that is more rapid than the advancement of height. The adrenal should be more suppressed in CAH and doses are usually in the range of 10–15 mg/m^2/day. Doses more than 15–17 mg/m^2/day in adolescence have been associated with reduced final height [23, 24].

The main differences between the various glucocorticoid preparations are their ratio of glucocorticoid to mineralocorticoid activity, their capacity to bind to various binding proteins, their molar potency and their biological half-life.

Hydrocortisone is a synthetic preparation of the steroid hormone cortisol. It has a quick onset of action, reaches the highest concentration usually around 2 hours after oral administration and is detectable in the circulation for 4–6 hours. It has an anti-inflammatory action, which usually lasts 6–8 hours.

Prednisolone, which has been modified slightly compared with hydrocortisone to prolong the duration of action, cannot be measured accurately in blood. It usually peaks at about 4 hours and lasts 6–8 hours; its anti-inflammatory action can last more than 12 hours.

Dexamethasone is structurally different and has a prolonged duration of action. It is significantly more potent than hydrocortisone. It also cannot be analysed in the blood. Its duration of action as an anti-inflammatory can be around 24 hours.

Dosing equivalents among glucocorticoids can be misleading because they relate to their anti-inflammatory properties for pharmacotherapeutic use rather than replacement therapy (Table 9.2). Differences in half-life, metabolism and protein binding cause significantly different growth suppressant effects compared with anti-inflammatory effects; prednisolone is estimated to be about 15 times more potent than hydrocortisone and dexamethasone 70–80-fold more potent.

The mineralocorticoid activities of glucocorticoid preparations also vary widely. Both glucocorticoids and mineralocorticoids can bind to both GR and MR. Mineralocorticoid activity is intimately related to the activity of 11β-HSD2 that metabolizes glucocorticoids but not mineralocorticoids to a form that cannot bind the receptor (Figure 9.9). Thus, the relative mineralocorticoid potency of various steroids is determined by both their affinity for the MR and their resistance to 11β-HSD2.

The plasma half-life and biological half-life of the various preparations can also vary widely. This is mainly related to binding proteins, hepatic metabolism and hepatic activation. Cortisone, which is widely used in some countries, and prednisone are biologically inactive until they are metabolized by hepatic 11β-HSD to their active forms, cortisol and prednisolone. This can complicate care in pediatrics as 11β-HSD has variable enzyme activity in childhood.

The route of steroid administration is also critical; orally administered steroids are absorbed incompletely, whereas IV and IM steroids are absorbed completely. The rate of absorption is more rapid for IV administration. Thus, if the secretory rate of cortisol is 8 mg/m^2 body surface area, the IM or IV replacement dose of cortisol would be 8 mg/m^2 but the oral dose would be about 15 mg/m^2, as only about half of an oral dose is absorbed intact. The efficiency of absorption of oral glucocorticoids varies considerably depending on factors including diet, gastric acidity and bowel transit time. Hydrocortisone suspension is not bioequivalent to hydrocortisone tablets and may give inadequate control due to an uneven distribution of the drug in the liquid. Crushing oral hydrocortisone tablets and mixing with a small volume of water immediately before administration is preferable to using a suspension.

Recently, an insulin pump has been used to administer hydrocortisone as a subcutaneous infusion in a small number of patients with poorly controlled glucocorticoid deficiency due to rapid clearance of hydrocortisone. This approach must be viewed as experimental and further research is required before it can be described as an acceptable modality of treatment [25, 26].

Modified-release oral hydrocortisone preparations have recently been developed that could improve cortisol delivery to mimic more closely physiology and have the potential to improve compliance as the frequency of dosing is reduced. Two formulations have been developed: Plenadren™ has a coating of hydrocortisone that is released rapidly followed by a slow release of hydrocortisone from the tablet matrix. Studies have shown improvements in metabolic profiles, weight, blood pressure and glycated haemoglobin in adults. Chronocort™ consists of a bilayer tablet with an insoluble coating protecting all but one face. The unprotected face has an outer non-drug layer that slowly erodes to expose the sustained-release hydrocortisone layer. This allows for a delayed release and a single cortisol peak early morning when administered once daily in the evening. Studies showed promising pharmacokinetic results and patients may benefit from twice-daily dosing but additional research is required, especially in young people. In addition, the length of the gastrointestinal tract and absorption characteristics are different in infants and children, so some of these adult-based formulations are not suitable. Studies are underway to develop more age-specific preparations [27, 28].

Mineralocorticoid Replacement

Children with PAI and confirmed aldosterone deficiency require treatment with fludrocortisone with a recommended starting dose of 100 μg (micrograms) daily. Infants also require sodium supplementation of 1–2 g/day (17–34 mmol/day, up to 10 mmol/kg/day) in several divided doses due to immature renal tubules and a reduced capacity to reabsorb sodium. All patients with elevated plasma renin concentrations or reduced aldosterone to renin ratios benefit from fludrocortisone therapy and adequate dietary sodium. Inadequate sodium supplementation is common and can also contribute to control overall, as it is more difficult to suppress ACTH if the salt balance is deranged. The tendency then is to increase glucocorticoid doses, which can have a detrimental effect on growth and metabolism. Salt supplements are generally not needed after infancy once a normal diet with cow's milk is introduced. Finally, sensitivity to mineralocorticoids increases with age and so requirement should be re-evaluated intermittently; fludrocortisone doses do not need to be increased greatly with age, unlike glucocorticoid doses.

Monitoring Treatment

How best to monitor replacement or suppressive treatment is a controversial area with different clinicians favouring different approaches, which may also depend on the underlying aetiology of the disease. Recommendations for monitoring patients with PAI but not CAH are primarily clinical and include assessment of growth velocity, weight, blood pressure and general clinical well-being including energy levels. Inadequate

weight gain, fatigue, anorexia and hyperpigmentation suggest a need for increased glucocorticoid dosing. Profiling of cortisol across a 24 hour period can be used to assess hydrocortisone absorption and clearance in certain settings.

The monitoring of children with CAH should include consistently timed hormone measurements in addition to clinical parameters including height, weight, physical examination for signs of virilization and regular bone age assessment after 2 years of age.

There are many options available for analysing the relevant hormone concentrations required to optimize CAH treatment including analysing blood, saliva, urine or dried filter paper blood samples. Cortisol profiles can be undertaken involving hospital admission for 24 hours to monitor cortisol and 17-hydroxyprogesterone concentrations on hydrocortisone treatment by regular blood tests through a cannula left *in situ*. Alternatively, salivary cortisol and blood 17-hydroxyprogesterone levels obtained on filter paper can be collected simultaneously by the patient at home. Androstenedione and testosterone concentrations may also aid in monitoring of treatment and can be useful in adolescents or young adults. Different institutions may favour one approach or a combination of methods.

ACTH is not very useful in monitoring the treatment of AI as aiming to consistently suppress ACTH will lead to overtreatment. The goal of monitoring steroid concentrations is not to aim for normality but to allow dose adjustments in the context of the overall clinical picture.

Routine adrenal imaging or bone mineral density evaluation is not recommended for children on physiological hydrocortisone replacement therapy.

Mineralocorticoid replacement therapy is monitored by clinical evaluation including weight gain, salt craving and dehydration, as well as biochemical analysis including blood electrolytes, PRA and aldosterone to renin ratio.

Glucocorticoid Withdrawal

Glucocorticoids have been used to treat virtually every known disease over the last 60 years. Currently their pharmacotherapeutic use is largely related to their anti-inflammatory properties but also their actions to lyse leukaemic leucocytes, increase mobility in Duchenne muscular dystrophy (DMD), lower plasma calcium concentrations and reduce increased intracranial pressure. There is only one major type of GR (GRα, NR3C1) that is present in most cells; therefore tissue-specific, disease-specific or response-specific analogues of naturally occurring glucocorticoids cannot easily be produced.

Pharmacological doses of glucocorticoids administered for more than 1–2 weeks can cause signs and symptoms of iatrogenic CS but mineralocorticoid effects are rare and there are no adrenal androgen effects. Alternate-day therapy can decrease the toxicity of pharmacological glucocorticoid therapy, especially suppression of the HPA axis and growth. Alternate-day therapy presumes that the disease state can be treated with intermittent therapy but this approach allows significant recovery of the HPA axis during the 'off' day.

When glucocorticoid therapy has been used for up to 10 days, therapy can be discontinued abruptly, even if high doses have been used. Although only one or two doses of glucocorticoid are needed to suppress the HPA axis, it recovers very rapidly from short-term suppression. When therapy has persisted for longer than 2 weeks, withdrawal with a tapered regimen of glucocorticoids is indicated to avoid a steroid withdrawal syndrome.

When reducing pharmacological doses of glucocorticoids, it is necessary to taper from the outset and not reduce the dosage precipitously to physiological replacement doses before tapering. Suddenly giving physiological replacement to patients who have been receiving pharmacological doses of glucocorticoids can lead to symptoms of glucocorticoid insufficiency and steroid withdrawal syndrome [16].

Procedures for tapering steroids are empirical. Their success is determined by the length and mode of therapy and by individual patient responses. Previous glucocorticoid therapy for several months will completely suppress the HPA axis but will not cause adrenal atrophy, whereas therapy of years' duration may result in almost total atrophy requiring a withdrawal regimen that takes months or even years.

In patients on long-standing therapy, a 25% reduction in the previous level of therapy is generally recommended weekly. When withdrawal is carried out with steroids other than cortisone or cortisol, measurement of morning cortisol values can be useful to indicate a recovering axis.

Even after successful discontinuation of therapy, the HPA axis may not be capable of responding to severe stress for 6–12 months. Evaluation of the axis using an ACTH stimulation test should be performed after a withdrawal programme to confirm adequate recovery and 'stress dose' replacement recommended if the child is unwell before full recovery has occurred.

Secondary AI

Secondary AI results from impaired ACTH secretion due to hypothalamic–pituitary (corticotroph) dysfunction. Several congenital and acquired causes are known, which are shown in Table 9.3 and discussed in more detail in Chapter 5. In secondary AI, ACTH concentrations are generally low or within the normal range;

however, in the context of hypocortisolaemia, the ACTH is inappropriately low. Mineralocorticoid synthesis is generally unaffected although mild hyponatraemia can occasionally be seen at diagnosis. Treatment of the adrenal component of these conditions is generally with standard replacement doses of glucocorticoids.

Isolated ACTH Deficiency

Isolated ACTH deficiency is a well-established but rare diagnosis, which can occur as part of a more complex syndrome or as a solitary event.

Pro-Opiomelanocortin (POMC) Deficiency

Defects in POMC affect the synthesis and release of ACTH as well as other cleaved peptides such as α- and β-MSH and β-endorphin (Figure 9.10). Because of the key regulatory role of these peptides in appetite regulation and skin/hair pigmentation, patients with POMC deficiency have secondary AI together with hyperphagia and rapid-onset obesity from infancy, pale skin and red hair. Children with naturally pigmented skin may show subtle changes of their hair roots.

Most pathogenic variants affecting POMC are nonsense or frameshift mutations that disrupt the entire gene and protein but several people with a similar phenotype (AI, obesity, pale skin, red hair) have been reported who have *elevated* ACTH concentrations. These patients harbour point mutations in the POMC protein (p.Arg145Cys) that correspond to a change in the cleaved ACTH peptide (p.Arg8Cys) causing *bioinactive* ACTH. Therefore, high concentrations of ACTH were measured but there was a functional ACTH insufficiency.

Treatment of the adrenal component of POMC deficiency is with standard glucocorticoid replacement. Recently, a melanocortin 4 receptor (MC4R) agonist, setmelanotide, has been used successfully to suppress appetite and cause dramatic weight loss in this condition (see Chapter 18).

Prohormone Convertase 1 (PC1) Deficiency

Defects in the enzyme PC1 (encoded by *PCSK1*) prevent the effective cleavage of POMC into sub-peptides as well as the cleavage of several other prohormones. Because of this aberrant processing, individuals with PC1 deficiency have secondary AI, hyperphagia and obesity, hypogonadotropic hypogonadism (HHG), abnormal glucose homeostasis with elevated proinsulin and low insulin and persistent diarrhoea.

TPIT (TBX19)

TPIT (encoded by *TBX19*) is a transcription factor that regulates POMC expression in the pituitary corticotrophs (Figure 9.10). Mutations in *TPIT* cause isolated ACTH deficiency but these children do not have obesity or altered skin/hair pigmentation because the defect is localized to the pituitary gland.

Mutations in *TBX19* usually cause severe secondary AI in the first few months of life. Approximately 65% of infants with severe neonatal isolated ACTH deficiency have homozygous or compound heterozygous mutations in TPIT. Infants usually have profound hypoglycaemia and hypoglycaemic convulsions are common. More than half the patients have prolonged cholestatic jaundice and the condition can be fatal if not recognized and treated promptly. *TBX19* mutations have not been reported in children with partial isolated ACTH deficiency or with milder childhood-onset forms. The molecular basis of these conditions is unknown.

Congenital Multiple Pituitary Hormone Deficiency

ACTH insufficiency can occur as part of a multiple pituitary hormone deficiency. Several genetic causes are established (e.g. *HESX1*, *OTX2*, *GLI2*, *SOX3*, *LHX3*, *LHX4* and *PROP1* mutations) but in many patients, the cause is not known (see Chapter 5). In some conditions the ACTH insufficiency may occur with time, some years after the onset of other pituitary hormone effects (e.g. *LHX3*, *PROP1*, growth hormone deficiency [GHD] type 2 due to *GH1* mutations). Therefore, careful long-term monitoring is needed and establishing a genetic diagnosis can be useful.

Secondary AI is an important diagnosis to make, especially in infancy, as hypocortisolaemia increases the risk of hypoglycaemia associated with GHD. Careful monitoring of fluid balance is also needed when hydrocortisone treatment is started as this may increase free water clearance and 'unmask' diabetes insipidus. Furthermore, cortisol can increase thyroid hormone metabolism and hypothyroidism can affect glucocorticoid metabolism, so careful monitoring of both is needed whenever thyroid or glucocorticoid replacement is started.

Acquired Multiple Pituitary Hormone Deficiency

ACTH insufficiency can also occur as part of an acquired multiple pituitary hormone deficiency; it can occur as a presenting feature of hypothalamic–pituitary tumours (e.g. pituitary, craniopharyngioma) or after neurosurgery, trauma (e.g. head injury, interrupted pituitary stalk syndrome) or high-dose cranial irradiation (see Chapter 5). In most situations, ACTH is the least likely pituitary hormone to be affected but a progressive decline in corticotroph function can occur with time, so long-term monitoring is needed. Similar caveats apply regarding glucocorticoid replacement.

ACTH Suppression

A relative suppression of ACTH response has been reported in several infections (e.g. HIV) and inflammation and, paradoxically, in association with chronic

stress, but by far the most common cause of ACTH suppression is exogenous glucocorticoid treatment for other conditions (iatrogenic).

Glucocorticoids and their therapeutic derivatives are widely used and, in many conditions, glucocorticoids with high efficacy are given orally for prolonged periods of time. Inhaled steroids for asthma (especially fluticasone) can have suppressive effects, as can potent eczema treatments (especially if applied to broken skin) and even topical eye drops (especially dexamethasone). Many of these treatments are instigated and monitored outside the remit of an endocrine clinic and issues can arise if the child is acutely unwell while on treatment and cannot mount an adequate stress response or in the period when the steroids are stopped or weaned. An overview of glucocorticoid withdrawal is given in 'Glucocorticoid withdrawal' section.

Causes of Primary AI (Excluding CAH)

An overview of causes of PAI is shown in Table 9.3 and monogenic causes in Table 9.4 [29].

Adrenal Hypoplasia

Adrenal hypoplasia is a congenital underdevelopment of the adrenal glands. Several well-established causes have emerged in recent years, often resulting from disruption of key transcription factors or cell cycle/growth regulators (Table 9.4). Most individuals develop early-onset salt-losing AI but milder or variable forms of these conditions exist. Treatment of adrenal hypoplasia is with standard replacement doses of glucocorticoids and mineralocorticoids with salt supplementation in the first year of life. Close attention is required to potential associated features.

X-linked Adrenal Hypoplasia Congenita (AHC) (NR0B1/DAX-1)

X-linked AHC was first reported in 1948 and described as 'cytomegalic' adrenal hypoplasia due to the presence of large fetal adrenal-like cells. As boys with this condition survived into adulthood following the introduction of glucocorticoid treatment, it emerged that HHG was an associated feature and that inheritance occurred in an X-linked pattern. In the 1980s the condition was mapped to the short arm of the X chromosome (Xp21) as it can occur as part of a contiguous gene deletion syndrome with glycerol kinase deficiency and DMD. In 1994, the gene responsible for X-linked AHC was reported as DAX-1 (officially known as *NR0B1*).

DAX-1 is an 'orphan' nuclear receptor. The C-terminal region of the protein resembles the LBD of nuclear receptors, although no known ligand exists. The N-terminal region consists of a repeat motif structure. Many studies have shown that DAX-1 acts paradoxically as a repressor of gene transcription through interactions with the related nuclear receptor SF-1 (NR5A1), although it may have an activator function in some contexts. Some researchers have suggested that the main role of DAX-1 is regulating differentiation of progenitor cells; a loss of DAX-1-mediated repression results in premature differentiation of these cells without prior expansion of cell numbers, resulting ultimately in organ hypoplasia.

The classic form of X-linked AHC is characterized by three main features: PAI, HHG and infertility. Forty percent of boys with X-linked AHC develop salt-losing PAI in the first 2 months of life. Others who do not present early develop AI insidiously throughout childhood (2–10 years). Most boys present with glucocorticoid and mineralocorticoid insufficiency and can sometimes be misdiagnosed as having CAH (21-OHD). Others initially show isolated mineralocorticoid insufficiency or predominant glucocorticoid deficiency and might be misdiagnosed as having aldosterone synthase deficiency or a form of FGD. Milder forms of X-linked AHC may first present with later-onset AI in teenage years or young adulthood [30].

The HHG associated with X-linked AHC probably represents a combined hypothalamic and pituitary defect. Many boys do not enter puberty but some have arrested pubertal development around Tanner stage 3/6–8 mL testes. An increasing number of reports have described evidence of exaggerated or early sexual maturation in X-linked AHC; this can present as macrophallus at birth or as early puberty in mid-childhood that subsequently arrests. Several reports suggested that this is gonadotropin independent and possibly driven by elevated ACTH but others have reported early puberty in boys who are receiving glucocorticoid treatment, so it may be a genuine DAX-1-related event. Men with late-onset X-linked AHC tend to have partial HHG.

Most men with X-linked AHC are infertile due to defects in spermatogenesis. Oligospermia has been seen in late-onset X-linked AHC. The response to fertility induction with gonadotropins is generally poor but this has been successful when combined with testicular sperm extraction (TESE)–intracytoplasmic sperm injection (ICSI) in one individual with classic early-onset X-linked AHC.

More than 300 patients and families with X-linked AHC have been reported. Most have nonsense or frameshift mutations in DAX-1/*NR0B1* or missense mutations clustered in the LBD region. One-sixth of patients have an isolated deletion of the gene and one-sixth have a contiguous gene deletion syndrome of Xp21. The features of this depend on which genes are deleted and the extent of the deletion: important centromeric genes include ornithine transcarbamylase (*OTC*), glycerol kinase (*GK*) and Duchenne muscular dystrophy

(*DMD*), so these conditions should be considered if there is a *NR0B1* deletion and assessed by urinary glycerol and serum creatine kinase. Very occasionally, a telomeric extension can include *ILRLAP1*, associated with X-linked developmental delay.

Genetic testing is extremely valuable to confirm the diagnosis or to make the diagnosis in boys with PAI of unknown aetiology. If there is a family history of AI in brothers or maternal uncles and evidence of HHG, the diagnosis of X-linked AHC is invariably made but, in countries with low consanguinity, the diagnostic yield for DAX-1/NR0B1 in male infants with salt-losing AI is also as high as 40% if other common conditions such as CAH have been excluded.

Given the X-linked nature of this condition, a detailed family history should be taken for AI or unexplained death among males on the mother's side of the family. The risk of brothers being affected or sisters being carriers is 50 : 50. Although second-born boys in a family tend to get diagnosed earlier, all at-risk individuals should be screened for AI or offered genetic testing as they may be pre-symptomatic. Early intervention can prevent a salt-losing adrenal crisis. Girls and women carriers can very occasionally manifest mild forms of X-linked AHC due to extreme skewed X chromosome inactivation.

Management of X-linked AHC includes replacement doses of glucocorticoids and mineralocorticoids as appropriate and monitoring and treatment of any associated pubertal disorder in a timely manner. It is currently unknown whether earlier use of follicle-stimulating hormone is of benefit in the long-term if assisted reproductive techniques are to be used (see Chapter 7). Some families and young persons may find psychological support useful to discuss issues of puberty and fertility. Any features of a contiguous gene deletion syndrome, especially DMD, need early diagnosis and support of a specialist team.

Steroidogenic Factor 1 Related (NR5A1/SF-1)

SF-1 (encoded by *NR5A1*) is a nuclear receptor that plays a key role in adrenal and reproductive development and function, as well as the expression of steroidogenic enzyme genes. Deletion of the gene encoding Sf-1 in the mouse results in adrenal and gonadal dysgenesis, variable HHG, abnormalities of the ventromedial hypothalamus and late-onset obesity. Therefore, SF-1 is considered a 'master regulator' of adrenal and reproductive biology as well as some aspects of metabolism.

In humans, disruption of SF-1/NR5A1 has been reported in only four children with AI. Two were 46,XY females with salt-losing AI and gonadal dysgenesis (with Müllerian structures) and two were 46,XX females with PAI. Most of these changes affected key amino acids in the DNA-binding region of SF-1, such as heterozygous changes in the P-box region (the main DNA-binding motif) or homozygous changes in the A-box region (an accessory DNA-binding motif) but systematic analysis has shown that disruption of SF-1 in children with otherwise undiagnosed PAI is relatively rare (~1%).

Heterozygous loss-of function variants in SF-1 have been reported in more than 200 individuals and families with a range of reproductive/gonadal phenotypes (see Chapter 4). These conditions include 46,XY DSD (due to testicular dysgenesis or impaired androgen synthesis), severe hypospadias, male factor infertility, primary ovarian insufficiency and even the development of ovotestes or testes in children with a 46,XX karyotype. Inheritance can be *de novo* dominant, sex-limited dominant or rarely recessive. It is unknown whether PAI may occur with time in these situations; current data suggest that this is not common but long-term follow-up will be needed [30].

IMAGe Syndrome (CDKN1C)

IMAGe syndrome, first reported in 1999, is characterized by *i*ntrauterine growth restriction, *m*etaphyseal dysplasia, *a*drenal hypoplasia and *ge*nitourinary anomalies (usually mild hypospadias or chordee). The adrenal component can be variable and diabetes mellitus has been reported in some individuals. IMAGe syndrome usually results from heterozygous pathogenic variants in the PCNA-binding domain of the cell cycle regulator CDKN1C. These changes cause a gain of function and growth repression.

An autosomal recessive form of IMAGe syndrome with immunodeficiency (POLE1) has also recently been reported.

CDKN1C is a paternally imprinted gene expressed from the maternal allele. Therefore, the condition is expressed only when the mutant allele is inherited from the mother. In familial cases, this can mimic X-linked inheritance but both boys and girls can be affected [31].

MIRAGE Syndrome (SAMD9)

SAMD9-associated conditions were first described in 2016 and more than 20 children have been reported. The condition is also known as MIRAGE syndrome. The key features are *i*nfections, intrauterine growth *r*estriction, *a*drenal hypoplasia, *g*onadal anomalies and *e*nteropathy. Most infants are delivered preterm due to severe growth restriction. AI can develop in the first days of life and severe hypospadias or female-typical genitalia can be seen in 46,XY children. Other features include patchy lung changes and respiratory distress, anaemia, thrombocytopaenia and hydrocephalus (possibly secondary to viral infections). Mortality is high. As some of these findings can be prevalent in preterm growth-restricted

infants anyway, it is probable that many babies with this condition die without the diagnosis being made, especially if the adrenal defect is not diagnosed and treated.

MIRAGE syndrome is caused by gain-of-function mutations in *SAMD9*. Usually these are heterozygous *de novo* variants, although germline transmission (with affected siblings) has been reported. SAMD9 may be involved in endosome function and recycling growth factor receptors, so gain-of-function changes result in severe growth restriction. *SAMD9* is located on the long arm of chromosome 7 (7q21). Children with *SAMD9* mutations who survive early infancy tend to develop monosomy 7 or partial 7q deletions, which remove the mutant allele and confer a clonal growth advantage on those cells. In the bone marrow, a loss of 7q21 (including *SAMD9* and *SAMD9L*) results in myelodysplastic syndrome, the 'M' in MIRAGE. Early detection is important as this is a preleukaemic state and bone marrow transplantation can be beneficial, but sometimes the bone marrow can recover spontaneously by revertant mosaicism. Other molecular mechanisms such as somatic loss-of-function mutations can also arise to ameliorate the growth-restricting effect of SAMD9. These dynamic changes may modify the phenotype in different organs so that some children have a mild or no adrenal component [19].

SERKAL Syndrome (WNT4)

SERKAL syndrome has been reported in one family and includes '*sex r*eversal' (46,XX testes), *k*idney anomalies, *a*drenal hypoplasia and *l*ung defects. This is usually lethal in early life and has been associated with homozygous disruption of WNT4.

Idiopathic (Unknown)

Adrenal hypoplasia may also occur where no cause is currently found using standard approaches.

Familial Glucocorticoid Deficiency (FGD)-Like Conditions (ACTH Resistance)

FGD, also known as isolated glucocorticoid deficiency or hereditary unresponsiveness to adrenocorticotropin (ACTH), is a genetically heterogeneous group of autosomal recessive disorders characterized by failure of the *zona fasciculata* cells within the adrenal cortex to respond appropriately to ACTH, resulting in AI with isolated glucocorticoid deficiency and elevated ACTH.

The archetypal forms of FGD involve ACTH resistance and defects in ACTH signalling because of disruption of the ACTH receptor (MC2R, FGD1) or the accessory protein needed for the receptor to localize to the cell membrane (melanocortin 2 receptor accessory protein [MRAP], FGD2); several other conditions can present in a similar manner and are sometimes considered *FGD-like* or *ACTH resistance-like* disorders (Figure 9.18). These conditions can be due to non-classic steroidogenic defects (*STAR*, CYP11A1/P450scc), alterations in oxidative stress pathways (nicotinamide nucleotide transhydrogenase [NNT], thioredoxin reductase 2 [TXNRD2], possibly AAAS), cell growth modifiers (MCM4) and novel metabolic processes (SGPL1) (Table 9.4).

Classic forms of FGD usually present with glucocorticoid insufficiency in early infancy or in childhood. The most common presenting features are those secondary to hypoglycaemia, including jitteriness, tremors, lethargy, poor feeding and hypoglycaemic seizures. In a small number of patients, undiagnosed hypoglycaemia in infancy may have been sufficiently severe to cause serious long-term neurological sequelae. Neonates may also present with jaundice, failure to thrive, collapse and very rarely transient neonatal hepatitis.

Older children present with a variety of features including increased pigmentation, recurrent infections, hypoglycaemia, lethargy and shock. As these are generally autosomal recessive disorders, there is sometimes a history of consanguinity and there may also be a history of unexplained neonatal or childhood deaths in the families.

Patients with FGD often have grossly elevated ACTH concentrations and can be hyperpigmented even at birth (Figure 9.17). This process is most likely due to stimulation of MC1R by POMC products (MSH) secondary to failure of the negative feedback loop to the pituitary and hypothalamus. MC1R is an important regulator of pigmentation in hair and skin and is responsible for the production of the darker eumelanin pigment. Genetic variants of MC1R are detected in most patients with fair skin but in <20% of patients with brown or black hair. Therefore the hyperpigmentation may be less common in patients from white ethnicities where loss-of-function variants in MC1R are more prevalent.

Mineralocorticoid production is usually preserved in patients with classic FGD and PRA and aldosterone concentrations are typically normal but rarely patients may have evidence of mineralocorticoid deficiency in unusually severe forms of the condition. Also, at presentation, children with FGD are usually stressed and may be hypovolaemic or pyrexial. They may be relatively water overloaded and mildly hyponatraemic because of reduced free water clearance associated with glucocorticoid deficiency. Therefore, some of these children may be misdiagnosed as having adrenal hypoplasia. Introduction of appropriate hydrocortisone replacement usually corrects minor electrolyte derangements and fludrocortisone replacement is not required but some other FGD-like conditions can have a genuine reduction in mineralocorticoid production in a subset of patients

due to biochemical blocks or disturbances in *zona glomerulosa* function (e.g. NNT, AAAS, SGPL1).

Treatment of FGD and related conditions is with physiological glucocorticoid replacement. ACTH concentrations often remain elevated in individuals who are receiving adequate replacement therapy and therefore cutaneous pigmentation can persist. It is important not to attempt to suppress the ACTH or use ACTH concentrations as a guide to replacement therapy as it will lead to overtreatment, potentially iatrogenic CS and poor growth.

MC2R (ACTH Receptor) (FGD1)

The first mutations in MC2R were discovered in 1993 in two siblings with FGD who had a homozygous mutation causing the amino acid change p.S74I. More than 40 pathogenic mutations in MC2R have now been reported. Most are missense mutations found as either homozygous or compound heterozygous changes. Missense mutations in MC2R are distributed throughout the receptor (Figure 9.19). Most mutations disrupt receptor trafficking to the cell surface, probably due to altered receptor folding during protein synthesis. Nonsense mutations are surprisingly uncommon and are usually associated with a missense mutation on the other allele. One exception is the c.560delT (p.Val187Alafs*29) variant found in Turkey and western Iran, which can be found as a homozygous change and is associated with severe early-onset disease, often with hypoglycaemic convulsions. Overall, *MC2R* mutations are found in ~25% of patients with FGD.

Some patients with FGD type 1 have tall stature for which the underlying mechanism is not clear. Since hydrocortisone replacement appears to stop excessive growth, it has been proposed that ACTH at very high concentrations may activate melanocortin receptors in bone and in the growth plate and stimulate growth. Alternatively, cortisol insufficiency may alter IGF-binding protein 5 (IGFBP5) dynamics in the osteoblast. Tall stature is not a recognized feature of FGD type 2, which tends to present at an earlier age than FGD 1, suggesting that the growth effects are due to prolonged exposure to high ACTH or low cortisol concentrations in childhood.

MRAP (FGD2)

MRAP was identified as a cause of FGD in 2005 following genetic linkage studies in families with FGD who did not have *MC2R* mutations. MRAP is a small single transmembrane domain protein essential for normal MC2R function. MRAP forms a unique antiparallel homodimer that directly interacts with the MC2R at the endoplasmic reticulum and is required for correct folding or trafficking of the receptor to the cell surface (Figure 9.18). Current evidence suggests that MRAP is also required at the plasma membrane for ACTH binding and signalling.

Mutations in *MRAP* are found in 20% of individuals with FGD. Most *MRAP* mutations that cause FGD result in either aberrant splicing or a severely truncated protein. Consistent with this, most patients with *MRAP* mutations present in the neonatal period with symptoms of severe cortisol deficiency, in contrast to patients with

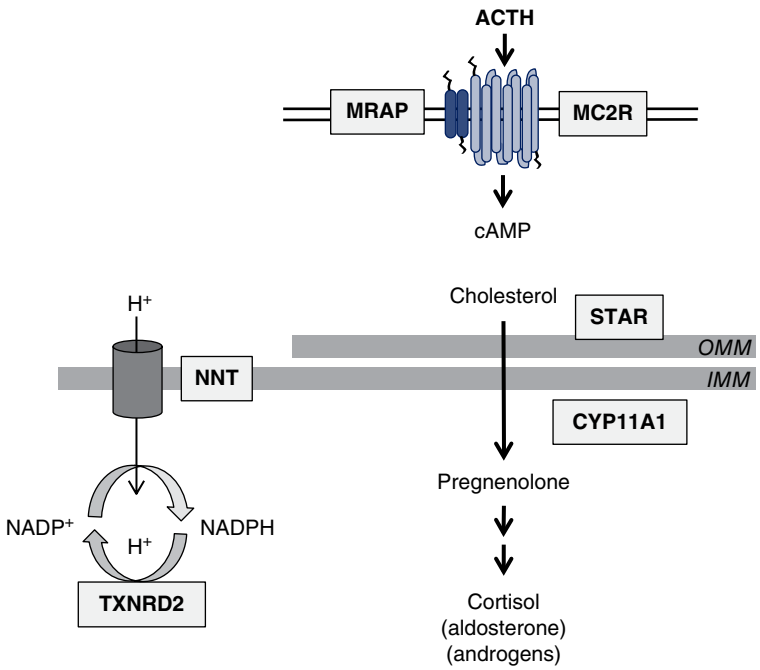


Figure 9.18 Cartoon showing the cellular function of several factors associated with familial glucocorticoid deficiency (FGD) and FGD-like phenotypes (MC2R, MRAP, STAR, CYP11A1, NNT, TXNRD2).

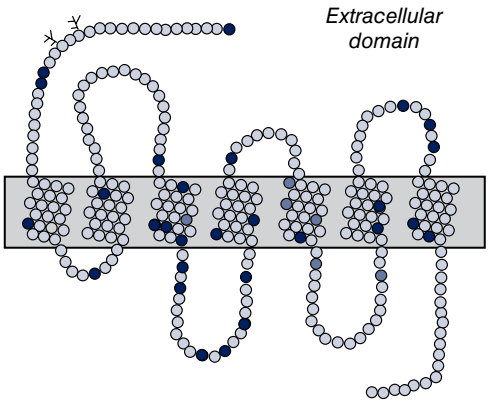


Figure 9.19 Cartoon showing selected pathogenic variants in the MC2R (ACTH receptor).

MC2R missense mutations who often present in the first few years of life. Several missense mutations in *MRAP* have also been reported, which give rise to a milder, later-onset form of the disease.

Non-Classic STAR *Insufficiency (Also Known as FGD3)*

StAR is a mitochondrial phosphoprotein that mediates the acute response to steroidogenic stimuli by increasing cholesterol transport from the outer to the inner mitochondrial membrane. Genetic mutations in StAR usually result in CLAH, a severe form of CAH. Patients with this condition usually present with a severe phenotype exhibiting glucocorticoid, mineralocorticoid and gonadal insufficiency. A small subset of patients presenting with FGD-like features were found to have non-classic mutations in StAR (Figure 9.18).

More than 40 mutations had previously been described in StAR, mostly concentrated in the C-terminal half of the protein encoded by exons 5, 6 and 7 in patients with CLAH. Most of them result in complete loss of function of the protein, whereas the mutations found in patients presenting with FGD (for example, p.Arg192Cys and p.Arg188Cys) had at least 20% partial function and only partial impairment of the cholesterol uptake function of this protein.

Mutations in StAR account for up to 5% of patients presenting clinically with FGD, although this is higher in consanguineous populations. Most individuals have isolated glucocorticoid deficiency with normal or near-normal renin and aldosterone levels but some patients do have mild reproductive anomalies including hypospadias and cryptorchidism, which had not previously been connected to their AI. The relative preservation of mineralocorticoid production probably reflects the lower production rate of aldosterone compared with cortisol, allowing the *zona glomerulosa* to avoid damage from lipid deposition. The long-term effects on sex steroids are unknown, although long-term monitoring of testosterone or oestrogens may be warranted and semen storage considered.

Non-Classic CYP11A1 Insufficiency

Like *STAR*, loss-of-function variants in cytochrome P450scc (*CYP11A1*) lead to adrenal and gonadal insufficiency but a number of patients have been reported with partial loss-of-function mutations and a milder clinical phenotype of isolated glucocorticoid deficiency consistent with FGD (Figure 9.18). Some patients also present with or develop mineralocorticoid insufficiency. The combination of a c.940G>A variant (rs6161) in compound heterozygosity with disruptive mutations is emerging as an especially frequent cause of this condition. Some boys have a history of delayed puberty but in general adult reproductive function is intact although long-term monitoring of sex steroids is warranted.

Oxidative Stress Pathways: Nicotinamide Nucleotide Transhydrogenase (NNT) and Thioredoxin Reductase 2 (TXNRD2)

Homozygosity mapping followed by targeted exome sequencing was used to identify mutations in NNT as another cause of isolated glucocorticoid deficiency in 2012. More than 20 mutations spread throughout the gene have been identified. They include abolition of the methionine translational start site, splice mutations and many missense and nonsense mutations. Defects in NNT are thought to be responsible for ~10% of cases of FGD. Presentation is usually between 6 months and 4 years of age. A subset of patients with severe disruption of NNT also develops mineralocorticoid deficiency as part of this condition and early puberty has been reported.

NNT is a highly conserved gene that encodes an integral protein of the inner mitochondrial membrane. Under most physiological conditions, the enzyme uses energy from the mitochondrial proton gradient to produce high concentrations of reduced nicotinamide adenine dinucleotide phosphate (NADPH) (Figure 9.18). The glutathione and thioredoxin systems require significantly high levels of this NADPH to detoxify reactive oxygen species (ROS) in mitochondria.

The finding of NNT mutations in FGD suggests that, at least in humans, NNT is of primary importance for ROS detoxification in adrenocortical cells. This hypothesis is supported by the further identification of mutations in TXNRD2 in a consanguineous kindred using whole exome sequencing. TXNRD2 is a human selenoprotein that also contributes significantly to redox homeostasis in the mitochondria (Figure 9.18). This single Kashmiri kindred had children presenting between 1 month and 10 years of age, sometimes also with congenital heart defects. Mineralocorticoid insufficiency was not present.

Oxidative stress impedes steroidogenesis and steroidogenesis itself induces oxidative stress because of electron leak throughout the steroidogenic pathway. The final step of cortisol production accounts for ~40% of the total electron flow from NADPH to detoxify ROS produced during steroidogenesis. This, together with the higher production of cortisol in comparison with aldosterone, may explain why the *zona fasciculata* is particularly susceptible to oxidative stress and why patients with *NNT* and *TXNRD2* mutations present primarily with isolated glucocorticoid deficiency rather than both glucocorticoid and mineralocorticoid deficiency. The identification of multiple defects in this pathway in patients with FGD highlights the susceptibility of the adrenal cortex to pathological damage by oxidative stress [32].

Minichromosome Maintenance 4 (MCM4)

A unique variant of FGD exists in the Irish travelling population, a genetically isolated population with high levels of consanguinity. Affected children develop hypocortisolaemia and compensatory elevated ACTH concentrations but retain normal PRA and aldosterone concentrations. Cortisol deficiency is frequently milder than in other forms of FGD and often occurs in childhood following a period of documented normal adrenal function. Patients can have several additional features including low birth weight and short stature, evidence of increased chromosomal breakage and natural killer cell deficiency with some patients demonstrating increased susceptibility to infection. Rarely, mineralocorticoid insufficiency can occur with time.

Targeted exome capture and high-throughput sequencing in a cohort of these patients identified a mutation in minichromosome maintenance-deficient 4 homologue (*MCM4*). MCM4 is one part of a heterohexameric MCM2-7 complex that acts as the replicative helicase and is essential for normal DNA replication and genome stability in all eukaryotes. This is the first association between a gene involved in DNA replication and AI. A MCM4-depleted mouse model showed abnormal adrenal morphology with steroidogenic cells displaced by non-steroidogenic capsular cells; if recapitulated in humans, this is likely to compromise adrenal steroidogenesis.

Depletion of MCMs has been proposed to lead to stem cell defects in mice. The relatively specific impingement of the MCM4 defect on adrenal function may be a consequence of its effect on the growth of adrenal stem or progenitor cells and their differentiation into steroidogenic cells. This suggests that MCM4 has additional functionality beyond DNA replication in adrenal development. This mutation in MCM4 is believed to be unique to the Irish traveller population and remains a rare cause of FGD [33].

Triple A Syndrome (AAAS)

AAAS consists of alacrimia (deficient tear production), achalasia (swallowing problems) and adrenal failure ('Addison disease'). Isolated glucocorticoid deficiency is seen in 80% of patients with additional mineralocorticoid deficiency present in a further 15%. Alacrimia and achalasia are seen in 80–90% of patients. A wide range of progressive neurological defects have also been associated with Triple A including motor, sensory and autonomic neuropathies, intellectual impairment and sensorineural deafness. Skin changes such as dermatitis can also occur. Adrenal impairment is rarely the presenting feature; affected children often first present with achalasia or developmental delay. There is little genotype–phenotype correlation and the clinical findings can be variable even within the same family.

AAAS is an autosomal dominant disorder but can resemble ACTH resistance as most affected individuals have isolated glucocorticoid deficiency. Oxidative stress, like NNT, has been implicated in the pathogenesis of AAAS. Mutations in *AAAS* lead to deficiency or mislocalization of the nuclear pore protein ALADIN, resulting in impairment of the nuclear import of DNA repair and antioxidant proteins making affected cells more susceptible to oxidative stress.

Sphingosine-1-Phosphate Lyase 1(SGPL1)

Several families and individuals have been reported with PAI and steroid-resistant nephrotic syndrome, who have been found to have homozygous or compound heterozygous mutations in *SGPL1*. In some children the adrenal disorder presented first, whereas the nephropathy was the presenting feature in others with the adrenal component delayed (sometimes due to the use of steroid treatment). Other clinical features included ichthyosis, primary hypothyroidism, neurological symptoms, lymphopenia, dyslipidaemia and cryptorchidism. Adrenal calcifications can be seen in some patients and some children have mineralocorticoid insufficiency.

SGPL1 is an enzyme that catalyses sphingolipid breakdown through cleavage of the lipid-signalling molecule sphingosine-1-phosphate (S1P). Mutations in other upstream parts of the pathway cause accumulation of lysosomal sphingolipid species and ceramide. These conditions are known as the sphingolipidoses and include Fabry disease, Gaucher disease and Niemann–Pick disease. The mouse model of *Sgpl1* deficiency has disordered adrenal zonation and defective steroidogenesis. This is an important diagnosis to make because of the potentially progressive multisystem effects of SGPL1 deficiency but the long-term natural history is currently poorly understood. In the future, specific therapeutic interventions targeted at this metabolic pathway could be developed [20].

Autoimmune Adrenalitis (Addison Disease)

Autoimmune adrenal disease (adrenalitis, Addison disease) in childhood can occur as part of an autoimmune polyglandular syndrome (type 1 or type 2) or as an isolated event. The age at presentation, associated features and causes vary depending on the underlying basis. Typical presenting features depend on the rate of onset of the condition and the degree of autoimmune destruction. Often there is a progressive and chronic pattern, resulting in weakness, anorexia, weight loss, fatigue, hypoglycaemia, frequent illnesses, hypotension and nausea. Hyperpigmentation is a common sign. More severe destructive events can cause mineralocorticoid insufficiency associated with hyponatraemia, hyperkalaemia and hypotension. Isolated mineralocorticoid insufficiency can sometimes occur at presentation or can develop following glucocorticoid insufficiency.

In some young people, autoantibodies can be detected, most often against P450c21 or P450scc. This finding may represent a secondary autoimmune event following destruction of adrenal tissue rather than the primary target for destruction. ACTH is elevated and cortisol response to Synacthen stimulation is impaired. Treatment is with standard replacement doses of glucocorticoids and mineralocorticoids, if needed. Assessment of potential associated features is necessary at the time of diagnosis. Long-term follow-up is needed to monitor for the emergence of mineralocorticoid insufficiency if not present initially and for the development of associated autoimmune features. DHEA(S) replacement has been proposed for some women if adrenal androgens are low, though the benefits are still unclear.

Autoimmune Polyglandular Syndrome Type 1

Autoimmune polyendocrine syndrome type 1 (APS-1) (also known as APECED or autoimmune polyglandular syndrome type 1) is a rare condition characterized by autoimmune Addison disease, chronic mucocutaneous candidiasis and hypoparathyroidism (see Chapter 11). The age of onset of these features can be very variable and at least two of the features are needed to make the diagnosis [34].

Autoimmune Polyglandular Syndrome Type 2

Autoimmune polyendocrine syndrome type 2 (APS-2) (also known as Schmidt syndrome) is the association of autoimmune adrenal disease with autoimmune thyroid disease and/or type 1 diabetes mellitus.

Isolated Autoimmune AI (Addison Disease)

Isolated (idiopathic Addison disease) is more commonly seen in 25- to 45-year-old adults, especially women (70%), and occurs in about 1 : 20,000 people. This condition is much less common in children but can occur in adolescents. The adrenal disease may be the first presentation of a polyglandular disorder, so long-term monitoring is needed for related autoimmune conditions, as well as for the emergence of mineralocorticoid insufficiency if not present at the time of diagnosis. Autoimmune adrenalitis is associated with specific HLA haplotypes (HLA-B8, HLA-DR3, HLA-DR4).

Several support groups are available worldwide for young people with autoimmune adrenal disorders and their families. These organizations can play an important role in education and in bringing young people with similar conditions together. Treatment has for many years focused on steroid replacement. Newer experimental approaches include immunomodulation, support of residual adrenal function and stem cell renewal but these are not yet widely adopted in clinical practice.

Metabolic Causes

Smith–Lemli–Opitz Syndrome

Smith–Lemli–Opitz (SLO) syndrome results from defects in 7-dehydrocholesterol reductase (DCHR7), an enzyme involved in cholesterol synthesis. Disruption of this enzyme can result in a reduction in cholesterol available for steroidogenesis. Other features include syndactyly between the second and third toes, proximal thumbs, typical facial features, microcephaly, cardiac anomalies and hypospadias in boys; although early-onset AI has been reported in some children with SLO, many children have preserved adrenal function or may have an attenuated stress response, especially if LDL cholesterol availability is low (e.g. inadequate diet, bile salt depletion). The diagnosis is made by finding elevated concentrations of 7-dehydrocholesterol (7-DHC) in the plasma and by genetic analysis of *DHCR7* [35].

Adrenoleukodystrophy/Adrenomyeloneuropathy

X-ALD is caused by mutations in a gene on chromosome Xq28, *ABCD1*. This gene encodes a peroxisomal membrane protein that belongs to the superfamily of ATP-binding cassette transporters. ABCD1 imports acyl-CoA derivatives of VLCFAs into peroxisomes, where β-oxidation shortens them. Affected individuals have a toxic accumulation of VLCFAs, which is associated with a pro-inflammatory state. Studies have shown increased levels of oxidative stress, protein damage, mitochondrial dysfunction and cell death in multiple tissues with VLCFA accumulation. This disorder is seen almost exclusively in males, with an incidence of about 1 in 16,000–20,000.

The mechanism of disease is unclear and there is no strong genotype–phenotype correlation; mild phenotypes can be associated with large deletions that abolish gene function and severe phenotypes associated with missense mutations in which abundant protein is

produced. The same pathogenic variant can be associated with significantly different phenotypes. It is therefore likely that single or multiple modifying genes contribute to the phenotype. Despite the variation in clinical phenotypes, nearly all males will have some neurological manifestation by adulthood and the biochemical phenotype of elevated VLCFA has nearly 100% penetrance.

There are three distinct clinical phenotypes. *Cerebral ALD* is a rapidly progressive inflammatory demyelinating disease with two peak times of onset, first between 4 and 8 years in 30–40% and second in adolescence or in young adult life in 20% of cases. Childhood patients develop normally but then present with declining behaviour and cognitive function, often manifesting as poor school performance. Subsequent features evolve as severe motor and sensory focal neurological deficits culminating in total disability in ~2 years and death within 4 years.

40% of patients present with *adrenomyeloneuropathy* (*AMN*), often aged 20–40. This subgroup of individuals presents with spastic paraparesis, sensory ataxia, sphincter dysfunction or impotence but disease severity and progression is highly variable. 10–20% of these patients develop cerebral ALD.

A further 10–20% of affected males present with AI, usually by the age of 7.5 years. Most of these patients develop AMN by middle age. Overall, adrenal function is abnormal in 90% of boys with neurological symptoms and in 70% of men with AMN. Adrenal monitoring in patients without AI is recommended every 6 months. Initially only glucocorticoid function may be affected but mineralocorticoid dysfunction will subsequently develop in at least 50% of patients. Adrenal antibodies are not present.

A small number of female carriers may also develop neurologic symptoms such as mild to moderate spastic paraparesis and, rarely, AI with time.

The diagnosis of X-ALD is confirmed by the characteristically high ratios of C_{26} : C_{22} and C_{24} : C_{22} VLCFAs in plasma and tissues, permitting diagnosis of carriers and affected fetuses as well as individual patients in 99% of cases. MRI is always abnormal in boys with neurological symptoms and may predate symptoms in some cases. Molecular genetic testing using sequence analysis confirms a pathogenic variant in 93% of patients and gene-targeted deletion/duplication analysis in 6% of cases. A newborn screening programme has been established in some states in the USA.

The only currently available treatment option for X-ALD is haematopoietic stem cell transplantation (HSCT) in boys with cerebral ALD; this has been shown to halt neuroinflammatory demyelination 12–18 months following transplant. It does not reverse any neurological deterioration and so timely recognition of cerebral involvement is essential. It does not help chronic myelopathy in AMN. Mortality rates in children are reported at 5–20% and in adults at 20–40%. Successful autologous HSCT using a lentiviral vector to transplant CD34+ with normal ABCD1 cDNA has been reported in two patients.

Treatment is largely supportive. Medication and physiotherapy may help with progressive spasticity. Dietary therapy with Lorenzo's oil improves circulating concentrations of VLCFAs but is ineffective in reversing established neurologic disease, but one open-label study reported prevention of cerebral disease. Current trials looking at antioxidant therapies are ongoing and thyromimetics, which increase ABCD2 (ALD-related protein) *in vitro*, are a possibility for the future [36].

Neonatal ALD is a rare severe infantile autosomal recessive form of ALD caused by mutations in one of the *PEX* genes encoding peroxins (e.g. *PEX1*/Zellweger syndrome spectrum). This defect also leads to elevated VLCFA but in contrast to X-ALD it presents much earlier, at birth or early infancy, usually with a very severe phenotype. Treatment is symptomatic.

Primary Xanthomatosis (Wolman Disease) and Cholesterol Ester Storage Disease

Primary xanthomatosis (Wolman disease) and cholesterol ester storage disease are two conditions that can occur due to defects in the secreted form of lysosomal acid lipase (cholesterol esterase), the enzyme that mobilizes cholesterol esters from lipid droplets in the adrenal gland. Disruption of this enzyme results in reduced availability of cholesterol for steroidogenesis and AI. The condition affects the ability of all cells to store and liberate cholesterol. Other features that develop in the first few weeks of life include failure to thrive, vomiting, steatorrhoea and hepatosplenomegaly. Calcification can be seen in the adrenal gland.

Wolman disease is characterized by the presence of foam cells containing large lysosomal vacuoles on bone marrow aspiration. These vacuoles contain cholesterol esters. Patient fibroblasts, bone marrow cells and leucocytes have deficient cholesterol esterase activity and genetic analysis of LIPA can be performed. Progressive clinical deterioration usually occurs and the condition is typically fatal.

Cholesterol ester storage disease appears to be a milder defect in the same enzyme generally presenting in childhood or adolescence. Few cases have been reported.

Mitochondrial Disorders

Mitochondrial function is dependent on two groups of factors: those that are encoded by genomic DNA in the cell and those encoded by the DNA of the mitochondrion itself. Mutations in mitochondrial DNA and

mitochondrial proteins can be associated with AI but the number of patients reported is small.

The most common defects involve large-scale deletions of mitochondrial DNA and include Kearns–Sayre syndrome (with progressive external ophthalmoplegia, retinitis pigmentosa, cardiomyopathy and heart block) or Pearson syndrome (with pancytopenia and exocrine pancreatic dysfunction). Genomic DNA-related autosomal recessive conditions reported with AI include defects in MRPS7/QRSL1 (impaired translation, with primary hypogonadism), NDUFAF5 (impaired complex I assembly) and GFER (impaired mitochondrial import, with congenital cataracts and lactic acidosis). Mitochondrial disorders should be considered when AI occurs with systemic features and the cause is not immediately clear [37].

Glucocorticoid Resistance (GR, NR3C1)

Defects in the GRα (encoded by *NR3C1*) cause familial glucocorticoid resistance (FGR) (also known as generalized glucocorticoid resistance or Chrousos syndrome). This condition can be inherited in a dominant or recessive manner. The clinical features are variable; patients often present with fatigue but other signs of glucocorticoid insufficiency are rare. Because the GRα defect interferes with central glucocorticoid feedback mechanisms, elevation of ACTH occurs to increase cortisol and compensate for the glucocorticoid resistance. The elevated ACTH subsequently increases adrenal mineralocorticoid and androgen secretion. This phenomenon explains the other common clinical and biochemical features of FGR, which include hypertension, hypokalaemia and metabolic alkalosis (mineralocorticoid effects), acne, male-pattern baldness, hirsutism, oligomenorrhoea and infertility (androgen effects especially noticeable in girls or women).

Most mutations in the GRα are missense changes located in the LBD. These variants tend to affect glucocorticoid binding or transactivation of target genes through altered nuclear localization or interactions with cofactors. Patients or carriers with heterozygous changes tend to have a milder phenotype, although several dominant-negative LBD variants (p.Ile559Asn, Ile747Met) have been reported that compromise function of the wild-type protein. Clinical and biochemical features in individuals with homozygous mutations in *NR3C1* are usually more severe. Treatment of glucocorticoid resistance includes mineralocorticoid-sparing synthetic steroids such as dexamethasone (1–3 mg/day) carefully titrated to suppress ACTH drive.

Disorders of Aldosterone Synthesis and Action

Aldosterone Synthase (CYP11B2)

Aldosterone synthase (P450c11AS, *CYP11B2*), a cytochrome P450 enzyme encoded by the *CYP11B2* gene, is exclusively expressed in the *zona glomerulosa* and catalyses the final three steps of aldosterone biosynthesis. The first step is the 11β-hydroxylation of DOC to corticosterone, the second step is the 18-hydroxylation of corticosterone to 18-hydroxycorticosterone (18-OH), and the third step is the 18-oxidation of 18-OH to aldosterone; it is the sole enzyme required to convert DOC to aldosterone.

P450c11AS, located on the long arm of chromosome 8, is the isozyme of P450c11β and is 93% identical in its amino acid sequence. Both P450c11AS and P450c11β are expressed in the *zona glomerulosa* and both can convert DOC to corticosterone but the conversion of corticosterone to 18-OH and subsequently to aldosterone is performed exclusively by P450c11AS and not two separate enzymes as previously thought.

Aldosterone synthase deficiency, also termed congenital hypoaldosteronism, is a rare inherited disorder transmitted as either an autosomal recessive or autosomal dominant trait with mixed penetrance caused by mutations in P450c11AS (*CYP11B2*). Aldosterone biosynthesis is impaired, while the *zona fasciculata* continues to produce corticosterone and DOC. This was previously termed 'corticosterone methyloxidase (CMO)' deficiency but is now termed aldosterone synthase deficiency and considered a spectrum of aldosterone deficiency depending on the nature of the P450c11AS gene defect. It is subdivided into two types – aldosterone synthase deficiency 1 (ASD1) and aldosterone synthase deficiency 2 (ASD2) – according to the relative concentrations of aldosterone and its precursors.

In both ASD1 and ASD2, corticosterone is increased and aldosterone decreased. ASD1 is associated with loss of both 18-hydroxylation and 18-oxidation enzyme activities (similarly to CMO I). Thus, the diagnosis for ASD1 deficiency is usually based on an increased ratio of corticosterone to 18-OH corticosterone. ASD2 results from amino acid replacement mutations in P450c11AS that selectively delete the 18-methyl oxidase activity while preserving the 18-hydroxylase activity. The diagnosis of ASD2 deficiency requires an increased 18-OH corticosterone with a low aldosterone concentration. The distinction between ASD1 and ASD2 is not precise and these disorders should be regarded as different degrees of severity on a clinical spectrum.

Aldosterone, under primary control of the renin–angiotensin system, regulates active sodium transport and excretion of potassium via the mineralocorticoid receptor in the distal convoluted tubules and renal collecting ducts. The absence of aldosterone biosynthesis will generally result in a salt-wasting crisis in infancy, at which time the normal secretory rate of DOC is insufficient to meet the newborn's mineralocorticoid requirements (like the newborn with P450c11β deficiency). Patients may present with a life-threatening salt loss or

failure to thrive, hyponatraemia, hyperkalaemia and metabolic acidosis in early infancy. The salt-wasting syndrome is typically less severe than in patients with 21-OHD or adrenal hypoplasia because of the persistent secretion of DOC. These patients may recover spontaneously and grow to adulthood without therapy; older children and adults usually have normal serum electrolytes, even if untreated. This probably reflects the increasing sensitivity to mineralocorticoid action with increased age, as reflected by the usual age-related decrease in serum aldosterone.

Mineralocorticoid Resistance (Pseudohypoaldosteronism Type 1) (MR, NR3C2)

Defects in the MR (encoded by *NR3C2*) are associated with a renal form of mineralocorticoid resistance known as autosomal dominant (or sporadic) pseudohypoaldosteronism type 1 (PHA I). Children with this condition usually present in early infancy with dehydration and failure to thrive. Biochemical tests show hyponatraemia, hyperkalaemia and elevated aldosterone concentrations and PRA. Some infants with elevated aldosterone and PRA remain asymptomatic and are only diagnosed by biochemical testing for other reasons. This is a relatively benign condition that can usually be adequately treated with sodium supplementation. The condition improves in childhood as mineralocorticoid sensitivity improves. This observation is in marked contrast to the autosomal recessive form of PHA I due to defects in the amiloride-sensitive ENaC. Autosomal recessive PHA I is a more severe systemic condition that does not improve with age.

Autosomal dominant PHA I results from heterozygous nonsense, missense and splice mutations in the MR. Missense mutations often affect key amino acids in the LBD and impair aldosterone binding, nuclear localization and aldosterone-dependent transactivation. Often changes arise *de novo* but some familial forms have been reported involving parents who either received treatment for the condition or who had a relatively asymptomatic course in infancy and survived without sodium supplementation.

Infections

AI can occur following specific infections or sepsis. Tuberculosis of the adrenal gland was a common cause of AI historically and still occurs. Fungal infections such as histoplasmosis and coccidioidomycosis are associated with adrenal dysfunction, as is bacterial sepsis (e.g. meningococcal, pneumococcal, streptococcal, haemophilus). HIV-associated AI is well recognized.

Haemorrhage

Adrenal damage due to haemorrhage is a feature of meningococcal septicaemia (Waterhouse–Friderichsen syndrome) and anti-phospholipid syndrome. Haemorrhage can also occur following trauma, especially if there is a bleeding tendency, or in the perinatal period. Idiopathic haemorrhage can also occur.

Infiltrative

Infiltrative disorders can damage the adrenal gland and cause AI. Neoplastic causes include tumour metastases as well as primary tumours (e.g. neuroblastoma). Amyloidosis, sarcoidosis and haemochromatosis can cause adrenal dysfunction but are rare in children.

Drug Effects

Although exogenous glucocorticoids are the most common cause of iatrogenic adrenal suppression, several other drugs cause adrenal dysfunction. They include ketoconazole, metyrapone and etomidate, which inhibit steroidogenic enzymes; the adrenolytic drug mitotane; and mifepristone, which interferes with GR function. In addition, glucocorticoid metabolism is increased by some anticonvulsants (e.g. phenytoin or phenobarbital) or antibiotics (e.g. rifampicin).

Idiopathic (Unknown)

Although a specific diagnosis can be reached in many children and young people with AI, sometimes the cause remains unknown. Some children have a transient AI in infancy that reverses. Occasionally, children have been started on steroid replacement because of a high suspicion of adrenal disease but without a full diagnostic evaluation and it emerges that their adrenal axis is intact once they are allowed to outgrow their glucocorticoid replacement and are retested.

Congenital Adrenal Hyperplasia (CAH)

CAH is the most common adrenal condition in children. In most countries, over 90% of CAH is due to 21-OHD. CAH affects ~1 in 13,000–18,000 children and can present with atypical genitalia and/or adrenal dysfunction at birth.

CAH is an umbrella term for defects in several enzymes involved in the steroidogenic pathway (Figure 9.6, Tables 9.3 and 9.4). The precise mode of presentation and clinical and biochemical features are determined by the nature and severity of the specific 'block'. In many ways, understanding the biology is straightforward; developing management plans that prevent the unwanted actions of hormones while maintaining adequate replacement is a challenge, especially on the background of dynamic changes across the 24 hours cycle, with age and with other physiological variables such as absorption, stress, illness and adherence to medication.

Congenital Lipoid Adrenal Hyperplasia

Steroidogenic acute regulatory protein (*STAR*) facilitates transport of cholesterol from the cytoplasm into the mitochondrion. *STAR* activity is necessary for steroidogenesis in the adrenal gland, testis and ovary, although a proportion of cholesterol transport is *STAR* independent.

Complete disruption of *STAR* activity causes CLAH, which results in severe defects in glucocorticoid and mineralocorticoid synthesis as well as in sex steroid production. Children with classic CLAH develop salt-losing AI in the first month of life, often between 2 and 4 weeks of age. All children have a female-typical appearance (46,XY and 46,XX) due to a block in fetal testosterone production but there are no Müllerian structures. Girls (46,XX) with *STAR* defects can enter puberty but tend to develop anovulatory cycles and can develop primary ovarian insufficiency.

The clinical features of CLAH may be explained by a 'two-hit hypothesis'. The primary event is a reduction in steroid synthesis resulting in an increase in stimulation by ACTH, angiotensin II or LH in the relevant cell type. This trophic stimulation increases cholesterol uptake into the cell, resulting in large lipid-filled vacuoles. The second hit results when the toxic free lipid results in cell death.

During early fetal development, the testis is stimulated by constant exposure to β-hCG rather than LH, so the fetal Leydig cells are damaged early and testosterone is not produced. This results in a lack of androgenization of the 46,XY fetus. Müllerian regression is unaffected as anti-Müllerian hormone (AMH) (Müllerian-inhibiting substance) is still produced by Sertoli cells, so no uterus or upper vagina is present, as is typical of all steroidogenic defects affecting 46,XY children (see Chapter 4).

The renin–angiotensin–aldosterone system undergoes significant stimulation only at birth; *STAR*-independent events can produce sufficient aldosterone initially and the secondary events take some time to manifest. Consequently, salt loss typically presents after several weeks and later than with other conditions such as CAH (typically 7–10 days).

Similar dynamics are important in the ovary. Steroidogenic pathways are relatively quiescent in the fetal ovary, so *STAR* defects have little effect. At the time of puberty, the ovary can synthesize limited oestrogen through *STAR*-independent pathways and at the start of each cycle, independent follicles are recruited. These can synthesize moderate amounts of oestrogen sufficient for breast development and growth but in the later stages of each cycle, the lipid accumulation is toxic so that progesterone synthesis is reduced and cycles are anovulatory. Primary ovarian insufficiency can sometimes occur with time and ovarian cysts can occur.

Many different homozygous and compound heterozygous mutations in *STAR* have been reported. The p.Gln258Ter nonsense mutation is especially prevalent in Japan and Korea and is carried by ~1 : 300 of the population, resulting in an incidence of CLAH of nearly 1 : 300,000 children. Other geographical hotspots include a p.Arg182His variant in Saudi Arabia, a frameshift in the Middle East and p.Leu260Pro in Switzerland.

Partial defects in *STAR* are associated with hypospadias and delayed-onset AI, whereas non-classic CLAH can present with isolated glucocorticoid insufficiency with no or only minor disruption of mineralocorticoid and sex hormone production. Non-classic CLAH is often associated with a p.Arg188Cys variant that affects lipid binding. This condition can resemble FDG and is discussed in 'Familial glucocorticoid deficiency (FGD)-like conditions (ACTH resistance)' section.

The diagnosis of CLAH/*STAR* deficiency is based on suspicion and supported by genetic analysis. Management includes standard replacement doses of glucocorticoids and mineralocorticoids with salt supplementation in infancy. Phenotypic girls with a 46,XY karyotype usually have gonadectomy and require oestrogen replacement for life if they are comfortable with a female gender. Gonadal carcinoma *in situ* has been reported. Close monitoring of oestrogen synthesis is needed for adolescent girls and women with classic CLAH and replacement given if required. Specialist input from fertility specialists may be needed. Boys with partial CLAH may sometimes require testosterone replacement to induce puberty and in adult life. Individuals with partial and non-classic CLAH should be monitored for the emergence of possible mineralocorticoid and sex steroid deficiencies with time; few long-term data are available for these patients.

P450scc (CYP11A1) Insufficiency

P450scc (encoded by *CYP11A1*) catalyses the first three enzymatic reactions needed to convert cholesterol to pregnenolone. This process is necessary for all aspects of steroidogenesis in the adrenal gland and gonad, including the production of progesterone, aldosterone, cortisol and sex steroids.

Originally it was felt that severe disruption of P450scc would be incompatible with survival in humans. This hypothesis came from the fact that higher primates such as humans rely on the placenta to produce progesterone to maintain pregnancy from around 4 to 6 wpc rather than the maternal corpus luteum (the so-called luteal-placental shift). As the placenta is mostly fetally derived, a complete block in P450scc would cause a lack of progesterone and loss of pregnancy.

Despite this hypothesis, several children have been described who have pathogenic variants in *CYP11A1*. In an early report, one child was born preterm with a family history of recurrent miscarriages but more recently several full-term babies with severe P450scc defects have been described. The typical presentation tends to be with hypoglycaemia and hyperpigmentation soon after birth with severe salt-losing AI at around the end of the first week. Infants with a 46,XY karyotype have a female-typical appearance but no Müllerian structures. Girls (46,XX) need oestrogen replacement at puberty, unlike CLAH. The c.835delA variant is found in northern Europeans but P450scc deficiency is a rare diagnosis.

As with CLAH due to *STAR*, a range of phenotypes can occur. Partial P450scc defects can present with delayed-onset AI and hypospadias in boys. Milder defects in the gene can present with a non-classic form of the condition that predominantly affects glucocorticoid synthesis and resembles FGD (see 'Familial glucocorticoid deficiency (FGD)-like conditions (ACTH resistance)' section).

The diagnosis of P450scc deficiency is based on suspicion and supported by genetic analysis. Treatment of P450scc-related conditions includes glucocorticoid, mineralocorticoid and sex steroid replacement as appropriate, with salt supplementation in infancy. The long-term effects of partial P450scc variants on mineralocorticoid and sex steroid synthesis are unknown and may need monitoring.

3β-Hydroxysteroid Dehydrogenase Type II (HSD3B2) Insufficiency

In the adrenal gland, 3β-HSD2 (*HSD3B2*) catalyses the conversion of Δ^5 steroids to Δ^4 steroids (i.e. pregnenolone to progesterone, 17α-hydroxypregnenolone to 17-OHP and DHEA to androstenedione) (Figure 9.6). Defects in this enzyme can affect mineralocorticoid, glucocorticoid and sex steroid synthesis but the clinical and biochemical picture can be complicated by the conversion of intermediate steroids to more active steroids by 3β-HSD *type I* in peripheral tissues.

Children with classic 3β-HSD2 deficiency usually present with atypical genitalia at birth. Boys (46,XY) tend to have severe hypospadias and a small phallus, glucocorticoid deficiency and variable degrees of salt loss. Girls (46,XX) can have clitoral enlargement as high concentrations of DHEA are converted to androgens, but some girls have normal genitalia. Milder forms of this condition can present with premature adrenarche in girls or with hirsutism and oligomenorrhoea.

The diagnosis of 3β-HSD2 can be made by measuring the ratio of Δ^5 to Δ^4 steroids following Synacthen stimulation or by a typical pattern on urine steroid profiling. It would be predicted that 17-OHP concentrations would be low due to the block but in fact these can be high due to peripheral conversion of 17α-hydroxypregnenolone to 17-OHP by 3β-HSD type I. Consequently, 3β-HSD2 can be misdiagnosed as 21-OHD or picked up in newborn 17-OHP screening programmes. Genetic testing is useful for confirming the diagnosis, especially in 46,XX girls with normal genitalia where the differential diagnosis can include many forms of AI. Sex steroid production may be insufficient at puberty.

Treatment includes glucocorticoids in doses to suppress any excess androgen production, as well as fludrocortisone and salt supplementation as needed. Close monitoring of puberty is needed and sex hormone supplements may be required.

17α-Hydroxylase/17,20-lyase (CYP17A1) Insufficiency

The enzyme P450c17 (encoded by *CYP17A1*) has both 17α-hydroxylase and 17,20-lyase activity and is a key branch point enzyme in steroidogenesis (Figure 9.6). The 17α-hydroxylation reaction can convert pregnenolone to 17α-hydroxypregnenolone and progesterone to 17α-hydroxyprogesterone (17-OHP). The C-17,20-lyase reaction primarily converts 17α-hydroxypregnenolone to DHEA in humans and requires cytochrome b5 (CYB5) and electron donors. Complete loss of P450c17 causes combined 17α-hydroxylase/17,20-lyase deficiency. This condition produces a block in glucocorticoid and sex steroid production. The ACTH drive results in an excess of DOC, which has mineralocorticoid effects and typically suppresses the endogenous mineralocorticoid pathway.

The classic presentation of children with complete forms of combined 17α-hydroxylase/17,20-lyase deficiency is a phenotypic girl (either 46,XX or 46,XY) who has absent puberty and who is found to be hypertensive, hypernatraemic and hypokalaemic due to DOC excess. Hyperpigmentation may be present but severe effects of glucocorticoid deficiency usually do not occur as a high molar concentration of corticosterone can have glucocorticoid activity. This is a potentially dangerous condition due to the blood pressure and electrolyte disturbances and cardiac arrhythmia can occur.

The diagnosis can be made based on a typical biochemical profile of hypernatraemia, hypokalaemia, mildly elevated ACTH, elevated DOC and corticosterone (if measured) with low PRA. The intermediate steroids rise on Synacthen stimulation but the cortisol response is inadequate. Urine steroid profiling has a characteristic pattern. Sex steroids (testosterone or oestrogen) are low and LH elevated if the child presents at the expected time of puberty. Ovarian cysts can occur. Partial forms of 17α-hydroxylase/17,20-lyase deficiency can present in 46,XY children with severe hypospadias

or with milder clinical phenotypes in 46,XX girls. Genetic analysis of *CYP17A1* is also useful.

Combined 17α-hydroxylase/17,20-lyase deficiency is rare and can be caused by a range of pathogenic variants in the gene. Genetic hotspots exist, especially in Brazil, with the p.Trp406Arg mutation found in patients of Spanish ancestry and the p.Arg362Cys found in those of Portuguese descent. Partial combined 17α-hydroxylase/17,20-lyase deficiency can sometimes be due to deletion of a phenylalanine residue at codon 53/54. Isolated 17,20-lyase deficiency has been reported that affects only sex steroid synthesis. This is difficult to diagnose biochemically and is often due to missense mutations that alter the distribution of surface charges in the redox binding site of P450c17 (e.g. p.Arg347His, p.Arg358Gln).

Treatment of combined 17α-hydroxylase/17,20-lyase deficiency is with suppressive doses of glucocorticoids and sex steroid replacement as indicated. Adjuvant treatment of hypertension may be needed.

P450 Oxidoreductase (POR) Insufficiency

POR (encoded by the gene *POR*) is a flavoprotein involved in electron transfer for microsomal cytochrome P450 enzymes. The main adrenal enzymes that require POR activity are P450c17 and P450c21, whereas others include aromatase, P450c51 and many hepatic enzymes involved in drug metabolism and detoxification.

PORD involves combined 17α-hydroxylase deficiency and 21-OHD, together with altered function of extra-adrenal enzymes. In many cases this results in the Antley–Bixler syndrome characterized by craniosynostosis, bowed femora, radio-ulnar synostosis/contractures, arachnodactyly, midfacial hypoplasia and choanal atresia. Genital anomalies are seen in 46,XY and 46,XX children. Variable glucocorticoid effects are seen but salt loss is uncommon. This contrasts with the form of Antley–Bixler syndrome caused by gain-of-function mutations in *FGFR2*, which is associated with normal steroidogenesis and normal genitalia.

The genital changes in 46,XY children probably reflect inadequate 17α-hydroxylase/17,20-lyase activity. The virilization seen in 46,XX children probably represents altered aromatase activity in the fetal placenta (resulting in exposure of the fetus to high concentrations of fetal adrenal androgens), as well as conversion of high concentrations of 17-hydroxyprogesterone to androgens through the 'backdoor' pathway of steroidogenesis. Children with PORD can have variable defects in sex steroid synthesis at puberty. Boys can have mild effects on testicular function and show spontaneous pubertal progression. Girls can develop hypogonadism and large ovarian cysts.

Some individuals with PORD do not have skeletal changes but present with reproductive anomalies alone, such as hypospadias (46,XY) or hyperandrogenism and cystic ovaries (46,XX).

More than 70 individuals with PORD have been reported. Most are homozygous for missense changes or compound heterozygous for missense and nonsense/frameshift variants. The p.Arg287Pro variant is especially common in Europeans, whereas the p.Arg457His variant is found in Japan. Different mutations can have different residual activity for 17α-hydroxylase/17,20-lyase and 21-hydroxylase reactions. Complete loss-of-function changes in POR have not been reported, suggesting that these may be embryonic lethal in humans, as they are in mice.

The diagnosis of PORD is supported by a skeletal phenotype but this is not always present. The biochemical profile can be very variable. Typically, cortisol concentrations are normal but have a poor response to ACTH stimulation. ACTH may be mildly elevated and 17-OHP can be raised and detected on newborn screening programmes. Steroids such as progesterone, corticosterone and 21-deoxycortisol can be elevated, whereas DHEA, androstenedione and testosterone can be within normal ranges or low. Urine steroid profiling shows a characteristic pattern of metabolites specific for PORD with increased excretion of the metabolites of progesterone (PD), 17-hydroxypregnenolone (5-PT) and 17-OHP (17HP, PT, P'TONE) as well as a mild increase in mineralocorticoid precursor metabolites (e.g. tetrahydrodeoxycorticosterone [THB]). Genetic testing of *POR* should also be performed.

Most children with PORD receive glucocorticoid replacement or doses to suppress ACTH drive. 'Stress' doses of steroids should be given for surgery or at times of illness as the cortisol response in these children is usually inadequate. Mineralocorticoid replacement is rarely needed. Sex steroids should be monitored and supplemented if required. Cystic ovaries can be difficult to manage and may require combined strategies with glucocorticoids and oestrogen. GnRH agonists have been used to suppress gonadotropin drive.

21-Hydroxylase (CYP21A2) Insufficiency

21-OHD results from mutations in the gene encoding P450c21 (*CYP21A2*). It is the most common cause of AI and accounts for up to 95% of CAH cases. 21-OHD is a challenging condition to manage as it requires a balance between replacing glucocorticoid and mineralocorticoids and suppressing sex steroids and other intermediate steroids while preventing the side effects of overtreatment. As increasing numbers of children with 21-OHD have reached adulthood, the lifelong

consequences of this diagnosis and its management need to be considered. Several consensus guidelines have been produced and an updated version of the Endocrine Society Clinical Practice Guidelines was published in 2018 [24].

Pathophysiology of 21-OHD

A complete block in P450c21 activity results in impaired aldosterone and cortisol synthesis, a reduction in central feedback and increase in ACTH drive and subsequent build-up of steroids higher in the pathway, which are shunted into the androgen pathway (see Figure 9.20).

The inability to convert progesterone to DOC leads to aldosterone deficiency. This results in severe hyponatraemia (sodium often below 110 mmol/L), hyperkalaemia (potassium often above 10 mmol/L) and acidosis (pH often below 7.1). The clinical signs are weight loss, hypotension, poor peripheral perfusion, shock, cardiovascular collapse and death, if not treated promptly. Fetal sodium and fluid balance is controlled by the mother's kidneys, so mineralocorticoid insufficiency starts to have an effect only after birth. At this time the newborn starts to lose whole body sodium but there is a delay in biochemical and clinical effects. Typically, progressive electrolyte disturbances occur from around days 4 to 5, sometimes with an initial rise in potassium before a fall in sodium, and clinical signs lead to presentation at around days 7–10. Weight loss occurs throughout this period, so a failure to recover early weight loss or a loss of more than 10% of birth weight may herald an impending salt-losing crisis.

The inability to convert 17-OHP to 11-deoxycortisol results in cortisol deficiency. This can result in dysregulated glucose metabolism and hypoglycaemia (although often less severe than in high blocks or adrenal hypoplasia). As cortisol has a facilitative effect on vascular tone, the cortisol insufficiency leads to a greater risk of cardiovascular collapse and shock. Unlike aldosterone,

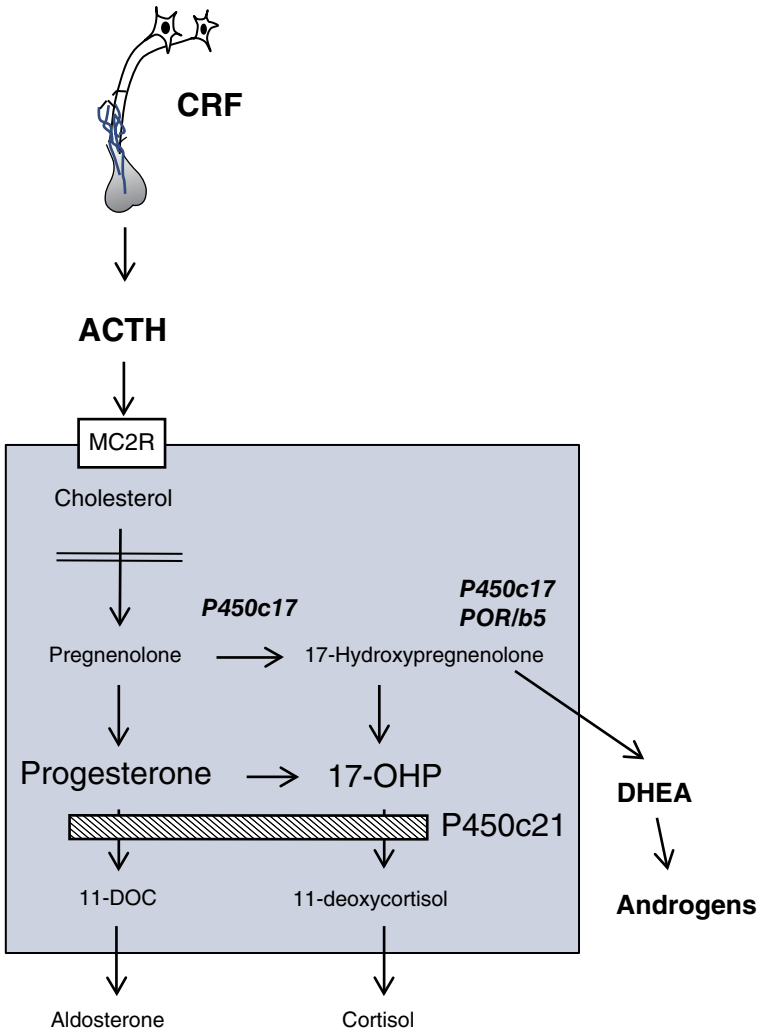


Figure 9.20 The biochemical consequences of 21-hydroxylase (P450c21) deficiency. The enzyme block is shown by the hatched bar. Recent data suggest that 11-oxygenated androgens such as 11-ketotestosterone are also generated by the adrenal gland in CAH, as well as "backdoor pathway" metabolites.

cortisol deficiency is present before birth and the ACTH drive is increased prenatally, sometimes resulting in hyperpigmentation at birth. This ACTH drive also leads to increased transcription of steroidogenic enzymes in the adrenal gland, further increasing the production of intermediary steroids proximal to the block (e.g. 17-OHP) and increased shunting of precursors into the androgen or alternative pathways. These precursors rise further once the baby starts to experience hypovolaemia after birth and ACTH drive increases to compensate.

In the 46,XY male fetus, the testes produce testosterone that causes development of the external genitalia from around 8 wpc. The excess of adrenal androgens in boys with 21-OHD does not seem to have an additional phenotypic effect. In contrast, the 46,XX fetus with 21-OHD is exposed to high concentrations of adrenal androgens in early development (in part through the 'backdoor' pathway and/or generation of 11-oxytestosterone), resulting in variable degrees of virilization of the external genitalia. As discussed previously ('Development and anatomy of the adrenal gland' section), the HPA axis feedback is intact in early fetal development (8–12 wpc), so low concentrations of cortisol lead to an increase in ACTH drive and adrenal androgen excess during this critical period in genital development.

Seventy percent of 46,XX children with 21-OHD present with atypical genitalia at birth. The genital changes associated with CAH include varying degrees of clitoromegaly/phallic growth, labioscrotal fusion, 'scrotalization' of the labia and urethral position; they are classified on the Prader scale (Figure 9.21, Table 9.5) (see Chapter 4). Children with Prader 4 or 5 genitalia are rare; these infants may have been assigned male at birth but have impalpable gonads. If these children are not monitored, they are likely to develop a severe salt-losing crisis several days later and could collapse and die. Therefore, the diagnosis of CAH (especially 21-OHD) needs to be considered in any apparent 'boy' with *impalpable* testis (not undescended testes), especially if a degree of hypospadias is present.

Moderate degrees of virilization result in 'ambiguous' genitalia typical of Prader 3. Often the phallus is bulky and rugose changes are seen in the labioscrotal folds. Internally, 46,XX children with 21-OHD have a uterus and ovaries and a common channel formed from the urogenital sinus that can vary in length. Postnatal androgens also influence the genital appearance; the size of the phallus can reduce once androgen production is suppressed. Conversely, girls with very poor control and high androgens can experience clitoral growth in childhood.

Clinical Forms of 21-OHD

21-OHD has a broad clinical spectrum that reflects, in part, the underlying gene mutation and its effect on the protein. Compound heterozygotes are very common, where two different mutations occur on different alleles. In these situations, the phenotype often reflects the *activity of the milder* change rather than that of the more severe change. Although three main subtypes of 21-OHD are usually considered (i.e. 'salt losing', 'simple virilizing' and 'non-classical'), in practice there is overlap between these categories.

Table 9.5 The Prader classification system for the appearance of external genitalia in CAH (46,XX).

Prader stage	Features
1	Mild clitoromegaly
2	Clitoromegaly, posterior labial fusion
3	Greater clitoromegaly, complete labial fusion, some 'scrotalization' of the labia, single perineal opening
4	Increased phallic size, complete labial fusion, 'scrotalization' of the labia, urethra-like opening at base or lower part of the phallus
5	Penis-like phallus, complete labial fusion, scrotal-like appearance of the labia, urethral meatus at the tip of the phallus

NB: In all situations, the gonads will be impalpable.

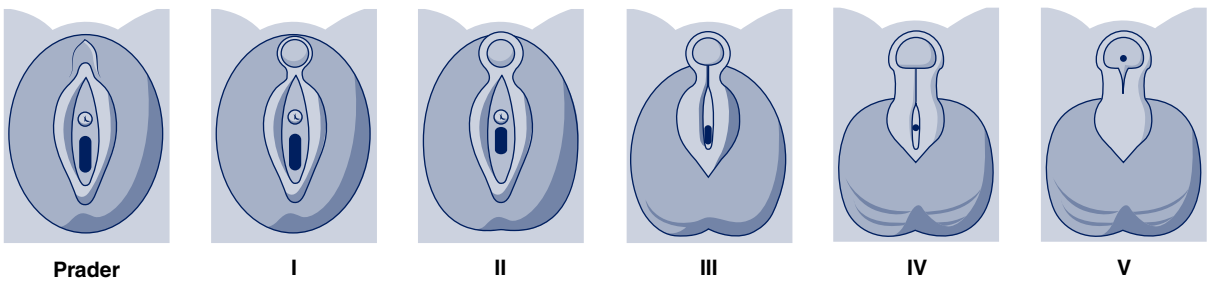


Figure 9.21 Prader staging of external genitalia in CAH.

Salt-Wasting 21-OHD 'Salt-wasting' 21-OHD results from complete deficiency of 21-hydoxylase activity affecting both mineralocorticoid and glucocorticoid activity. Boys (46,XY) usually present in a salt-losing crisis in the second week of life, as described above. Girls (46,XX) are usually identified at birth because of atypical genitalia and are at risk of a salt-losing crisis if not diagnosed and treated appropriately before it develops. Approximately 75% of 'classic' 21-OHD is salt wasting.

Simple Virilizing 21-OHD 'Simple virilizing' 21-OHD results from mutations that cause partial loss of 21-hydroxylase activity and accounts for ~25% of 'classic' CAH. Because the adrenal gland synthesizes much greater molar concentrations of cortisol compared with aldosterone, the main biochemical defect is glucocorticoid insufficiency and the mineralocorticoid pathway is somewhat spared, unless under stress (e.g. fluid restriction and salt deprivation). Boys (46,XY) with simple virilizing 21-OHD typically present in mid-childhood (3–7 years of age) with signs of hyperandrogenism, namely, pubic and axillary hair, phallic growth (but small testes) and increased growth rate with an advanced bone age. Girls (46,XX) with simple virilizing 21-OHD are usually identified at birth because of atypical genitalia but otherwise have growth acceleration and pubic hair. They do not develop a severe salt-losing crisis but the distinction between 'salt-wasting' and 'simple virilizing' forms of 21-OHD is not always clear and often children classed as 'simple virilizing' CAH benefit from treatment with fludrocortisone to obtain better control and avoid overtreatment with glucocorticoids (see treatment below).

Non-Classic 21-OHD A non-classic, milder form of 21-OHD is relatively common and sometimes called late-onset CAH. Children can have signs of hyperandrogenism, accelerated growth and earlier epiphyseal fusion. Women with this condition often have acne and hirsutism (60%). Other features include menstrual irregularities, polycystic ovaries, decreased fertility or frontal baldness. Sometimes there may be no phenotypic manifestations at all other than an increased response of plasma 17-OHP to an IV ACTH test. Mild impairment in mineralocorticoid secretion may be seen. Much less is known about the effects of non-classic 21-OHD in males; early facial hair growth and an enlarged phallus have been reported.

Contiguous Gene Deletion Syndromes Occasionally, 21-OHD can be part of a contiguous gene deletion syndrome that also affects other genes in the locus, most notably tenascin X. Loss of tenascin X causes connective tissue disorders; homozygous deletions can be associated with Ehlers–Danlos syndrome, whereas haploinsufficiency (one copy lost) causes joint hypermobility. These features should always be sought in individuals with 21-OHD.

Prevalence of 21-OHD

Based on screening programmes and national surveys, the prevalence of salt-wasting and simple virilising 21-OHD in many countries in Europe and North America is 1 in 14,000–18,000 live births. Approximately 1 in 60 people are heterozygous carriers. Although the frequency in Caucasians and Hispanics is similar, 21-OHD is 2–3 times less common in African Americans and much rarer in families of pure African ancestry. Yupik Eskimos of Alaska have the highest prevalence of 21-OHD at around 1 in 300 and in Saudi Arabia it is 1 in 5000.

Non-classic 21-OHD is much more common. The prevalence is ~1 in 27 for Ashkenazi Jews, 1 in 50 for Hispanics, 1 in 60 for Yugoslavs and 1 in 300 for Italians. In other Caucasian populations the prevalence is somewhat lower but there can be overlap between 17-OHP concentrations in non-classic CAH and heterozygous carriers of classic 21-OHD, so the exact prevalence may vary and require genetic testing and assessment of clinical features.

Genetics of 21-OHD

The gene that encodes 21-hydroxylase is *CYP21A2*. This gene is located on the short arm of chromosome 6 (6p) ~30 kilobases from a pseudogene (*CYP21A1P*) and within the HLA gene cluster. The pseudogene is inactive but also contains 10 exons and has high sequence homology with *CYP21A2* (98% between exons, 96% between introns) (Figure 9.22).

The genomic region encompassing containing *CYP21A2* and *CYP21A1P* contains several genes that are duplicated in tandem repeat. This phenomenon increases the risk of unequal crossover during recombination in meiosis, which can lead to deletion or duplication events and disruption of the gene. Another genetic event that can occur when a gene and pseudogene are in close proximity is 'gene conversion'. During gene conversion a segment of *CYP21A2* is replaced by a copied region of *CYP21A1P*. Consequently, the functional gene contains sequence variants from the pseudogene that make it biologically inactive.

An overview of some of the most common pathogenic variants in *CYP21A2*/21-hydroxylase is shown in Table 9.6. Approximately 20–30% of mutated alleles involve a 30 kb deletion of the 3′ end of *CYP21A1P*, the *C4B* complement gene and the 5′ start of *CYP21A2*, which produces a chimeric protein with no function (Figure 9.22). Other common variants affecting 20–30% of 21-OHD associated alleles are the c.293-13A > G or

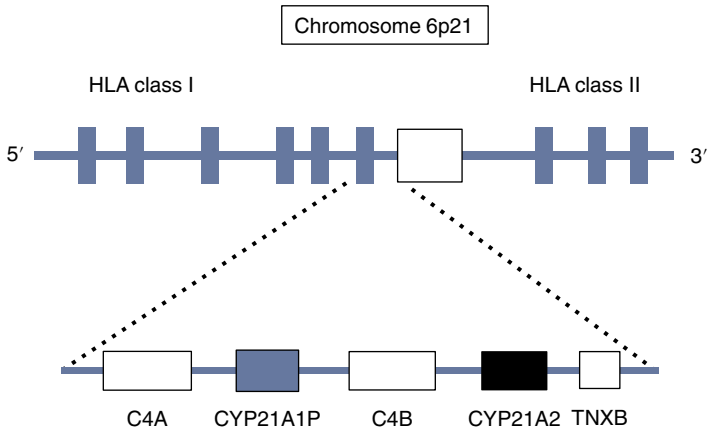


Figure 9.22 Genomic locus on chromosome 6p21 containing *CYP21A2* (encoding 21-hydroxylase), the *CYP21A1P* pseudogene and related genes such as tenascin X (*TNXB*) and the HLA class I and II antigens.

Table 9.6 A selection of the most common *CYP21A2* mutations found in 21-OHD.

Mutation	Enzyme activity (% of wild type)	Most common associated phenotypes
Gene deletion	0	Classic
Large gene conversion	0	Classic
p.Pro31Leu	30–60	Non-classic
c.293-13A>G	1	Classic
c.293-13C>G	1	Classic
p.Gly111ValfsTer21	0	Classic
p.Ile173Asn	3–10	Classic/simple virilizing
p.Ile237Asn/Val238Glu/Met240Lys	0	Classic
p.Val282Leu	20	Non-classic
p.Leu308PhefsTer6	0	Classic
p.Gln319Ter	0	Classic
p.Arg340His	20–50	Non-classic
p.Arg357Trp	2	Classic/simple virilizing
p.Pro454Ser	20–50	Non-classic

NB: Codon numbering may be different to historical publications (e.g. p.Ile173Asn was formally p.Ile172Asn; p.Val282Leu was formally p.Val281Leu).

c.293-13C > G changes that affect splicing and generally result in minimal residual activity.

Nine other common variants are non-functional changes in the pseudogene that can affect *CYP21A2* by gene conversion. Overall these changes, together with the deletions and splice variants described above, account for ~95% of pathogenic alleles. More than 100 other rare single nucleotide changes, small deletions/insertions and complex rearrangements have been described accounting for the remaining 5% of events. Large deletions of the locus can involve the tenascin X gene (*TNXB*) and cause a form of hypermobility (haploinsufficiency) or Ehlers–Danlos syndrome (homozygous) as a contiguous gene deletion syndrome (Figure 9.22).

Pathogenic variants/deletions in *CYP21A2* are inherited in an autosomal recessive manner. Therefore, both parents are carriers and there is a 1 in 4 chance (25%) of having an affected child. Some individuals have homozygous mutations, especially in regions where consanguinity is high, but most children have compound (double) heterozygous mutations; that is, different pathogenic variants are inherited from each parent, one on each allele. Approximately 1% of *CYP21A2* mutations arise *de novo*.

A reasonable genotype–phenotype correlation that is broadly related to the residual enzyme function of the protein was thought to exist for 21-OHD. An overview of some of the activities and most common phenotypes associated with different mutations is shown in Table 9.6. In compound heterozygous states, when different mutant

alleles are inherited from each parent, the phenotype often tracks with the milder change but this genotype–phenotype correlation is not invariable and a spectrum of phenotypes can be seen. A recent study has suggested that a direct genotype–phenotype correlation is seen in ~50% of cases. For example, the p.Pro31Leu and p.Val282Leu variants are usually associated with non-classic 21-OHD but can be found in classic 21-OHD in 2–3% of cases. Finally, a duplicated allele can have one wild-type (normal) copy and a mutated copy (often p.Gln319Ter), which can lead to difficulties in establishing carrier status and pathogenicity.

Although many changes in *CYP21A2* disrupt its function through deletion, nonsense or splicing events, the recurrent missense mutations in P450c21 can provide some insight into structure–function relations. For example, p.Arg357 may be part of the redox partner binding site, p.Cys429 lies within the haem binding site, p.Val282 is involved in haem structure, and p.Pro30 may be involved in membrane tethering and stability of the enzyme [7, 38].

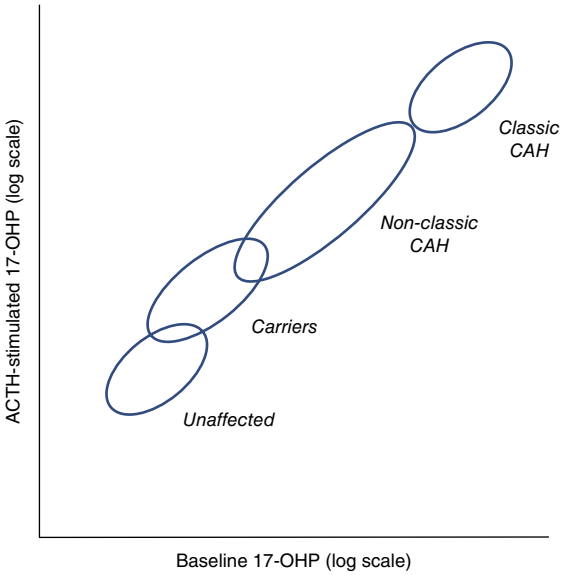
The Diagnosis of 21-OHD

The diagnosis of classic 21-OHD is suggested clinically by the presence of genital changes at birth, a salt-losing crisis in either sex or the development of increased growth and androgen excess (e.g. pubic hair, acne) in prepubertal boys or in girls with milder non-classic 21-OHD (see below). Plasma 17-OHP is the most useful marker and is usually very elevated and shows an exaggerated response following Synacthen stimulation (Figure 9.23) (see also 'Diagnosing the specific cause of AI' section). Concentrations can vary with age, prematurity and stress, so this needs to be considered when the sample is taken. Urine steroid profiling shows a typical pattern of steroid excretion and can differentiate other forms of CAH.

If this is not available, measurement of 11-deoxycortisol, DHEA and androstenedione can also be useful if another form of CAH is suspected. As noted previously, 17-OHP can also be elevated in several other forms of CAH (3β-HSD2, P450c11, PORD), with some adrenal and testicular tumours, in preterm infants and in the first 48 hours of life (unless measured by LC-MS/MS) and in severely unwell babies under stress. Basal cortisol is often within normal ranges in 21-OHD if measured by standard non-LC-MS/MS assay platforms but the response to Synacthen stimulation is usually attenuated. The role of screening programmes for CAH is discussed elsewhere ('Diagnosing the specific cause of AI' section).

PRA is usually elevated in severe forms of 21-OHD and aldosterone, if measured, is low. Children with 'simple virilizing' forms of CAH often have elevated PRA, especially if sodium restricted. Treatment with mineralocorticoid replacement can be an important adjuvant to reduced glucocorticoid doses and better control.

(a)



(b)

	Classic-21OHD	Non-classic 21-OHD	Unaffected
Basal 17-OHP	>300 nmol/L (>10,000 ng/dL)	6–300 nmol/L (200–10,000 ng/dL)	<6 nmol/L (<200 ng/dL)
Stimulated 17-OHP	>300 nmol/L (>10,000 ng/dL)	31–300 nmol/l (1,000–10,000 ng/dL)	<30 nmol (<1,000 ng/dL)

Non-classic basal values may be within normal range

Figure 9.23 Diagnostic value of 17-hydroxyprogesterone (17-OHP) in 21-hydroxylase deficiency (21-OHD). (a) Representative nomogram of baseline 17-OHP and ACTH-stimulated 17-OHP in individuals with classic and non-classic 21-OHD, carriers and unaffected individuals (shown on a $\log_{10}$ scale). (b) Table of typical 17-OHP values for each diagnostic category.

The diagnosis of non-classic 21-OHD is more difficult and requires demonstration of elevated 17-OHP with an exaggerated response following ACTH stimulation, as well as hyperandrogenism. There can be overlap between basal 17-OHP in non-classic CAH and heterozygous carriers of severely disruptive mutations (Figure 9.23).

Genetic testing can have an important role to play in making a diagnosis of 21-OHD or confirming a biochemical diagnosis. Direct sequencing of the gene is usually performed to investigate for point mutations such as missense variants, splice changes and nonsense mutations. Initial analysis can be focused on common pathogenic variants and extended to the whole gene if nothing is discovered. Approximately 70–80% of 21-OHD is due to single nucleotide variants. Analysis of deletions, duplications or micro-rearrangements requires alternative targeted

approaches such as quantitative PCR, long-range PCR, multiplex ligation-dependent probe amplification (MLPA) or specifically targeted microarrays. This approach is usually carried out if no or only one point mutation is found. Deletions and duplications account for ~20–30% of 21-OHD. In addition, genetic analysis should be offered to parents to understand the inheritance risks. Analysis of parental samples in cases of apparent compound heterozygosity is also important to show that the two pathogenic variants are in *trans* (i.e. on different alleles) rather than having multiple gene conversion events in *cis* (i.e. on the same allele) [39].

Treatment of 21-OHD

Treating patients with CAH involves the same principles required for treating any patient with AI (see 'Treatment of AI' section) but maintaining the delicate balance between overtreatment and undertreatment is even more critical and challenging (Figure 9.24). In contrast to other forms of AI, undertreatment also results in continued overproduction of adrenal androgens, which hastens epiphyseal maturation and closure, resulting in significantly compromised growth and other manifestations of androgen excess.

Treatment in childhood CAH is primarily with hydrocortisone in 3–4 divided doses daily but at a slightly higher maintenance dose of 10–15 mg/m^2/day due to the additional risks of undertreatment. Newly diagnosed patients, especially newborns, usually require substantially higher initial dosages to suppress their hyperactive CRH–ACTH–adrenal axis: simple physiological replacement is usually insufficient to suppress adrenal androgens. It is important that infants are reassessed frequently and the dose normalized as soon as possible to avoid prolonged overtreatment during a period when there is normally very rapid growth. Steroid suspensions should be avoided and crushed tablets are preferred. As with hydrocortisone replacement in general, a greater dose is usually given in the morning.

Control may deteriorate during puberty despite good adherence due to increased cortisol clearance; frequent reassessment is necessary. Although the main aim is to control hyperandrogenism, it is also essential to treat with the lowest dose possible to provide control as adult height correlates negatively with glucocorticoid dose in early puberty.

Mineralocorticoid replacement with fludrocortisone 50–200 mcg/day is required. In addition sodium supplements are necessary, usually in infancy, at a dose of 1–2 g/day (17–34 mmol/day, up to 10 mmol/kg/day) in several divided doses due to immature renal tubules and a reduced capacity to reabsorb sodium. All patients with elevated PRA or low aldosterone to PRA ratio will benefit from fludrocortisone therapy, which may help contribute to lower glucocorticoid doses and improve control.

Monitoring of treatment in CAH is difficult but should primarily be clinical including precise monitoring of growth and growth velocity, signs and symptoms of iatrogenic CS and hyperandrogenism. It should include regular bone age assessment and hormonal measurements including cortisol, 17-OHP, androstenedione and testosterone. Steroids can be measured by analysing blood, saliva, urine or dried filter paper blood samples and tests should be timed accurately in relation to hydrocortisone doses to enable dose adjustment.

Children with early puberty due to adrenal androgen excess are at risk of developing true central gonadotropin-dependent precious puberty once the adrenal androgens are controlled. GnRH agonists may be needed to prevent the development of central precocious puberty [24].

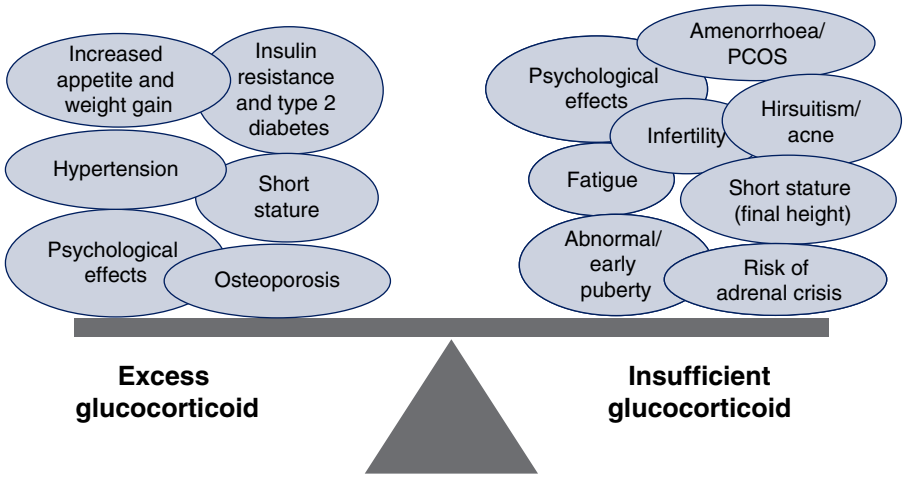


Figure 9.24 The balance of excess glucocorticoid treatment compared with insufficient glucocorticoid treatment in 21-hydroxylase deficiency (21-OHD).

Additional Treatments Specific to 21-OHD

Given the challenges in 21-OHD management, several different approaches to treatment have been used in the past and several novel approaches are under investigation.

Alternative steroids such as prednisolone and dexamethasone have been used. Both are long-acting steroids not recommended in children because of their growth-suppressing effects. Prednisolone has been used in adults to reduce the frequency of medication but can have greater side effects. Dexamethasone should be avoided.

Different timing of steroids has been tried. For example, extra doses in the night (3–4 am) have been used in 21-OHD difficult to control. Reverse dosing has also been tried, with the greatest dose given in the evening rather than in the morning to try to suppress ACTH. Both regimens result in sleep disturbances due to higher nocturnal steroid concentrations. Newer delayed-release preparation of hydrocortisone may provide a more physiological replacement and potentially better control but data in children are limited. Subcutaneous hydrocortisone pumps have also been used on an individual basis but extensive studies have not been reported and this approach should be considered experimental.

Other approaches have been used to try to limit effects of androgen excess and/or to promote growth. These include anti-androgens (e.g. flutamide), aromatase inhibitors or more targeted blockade of enzymes such as P450c17 by newer pharmacological agents (e.g. abiraterone). In addition, novel CRH antagonists are in development, which may permit lower doses of glucocorticoid while reducing the androgen excess. It is possible that combinations of therapies targeted at different aspects of control and modified for personal pharmacogenomics may represent the most promising approach in the future. Furthermore, gene therapy to correct the specific mutation causing CAH could lead to a potential cure. Unfortunately, though, current gene therapy techniques and issues with viral gene therapy vectors mean that this is not currently a viable treatment.

One final option to prevent the effects of 21-OHD is bilateral adrenalectomy. This is a radical surgical approach to eradicate adrenal androgen excess but is rarely carried out and is considered only in select cases, most often in women with salt-wasting CAH and infertility who are trying to get pregnant, as the high adrenal progesterone prevents ovulation. The risk for non-adherence is an important issue as there is a potential increased risk for adrenal crisis due to the loss of residual adrenal function; many of these individuals have issues with compliance already. In addition, loss of hormones such as adrenaline and DHEA may have detrimental effects.

The role of surgery in CAH and optimal timing is under ongoing debate. This is an extremely complex area that needs careful consideration and counselling by an experienced team [40, 41].

Surgery typically involves vaginal reconstruction (vaginoplasty/urogenital sinus mobilization) and, in some situations, corporal (clitoral) reduction [40, 41]. The aim of vaginal reconstruction is to allow penetrative sexual intercourse/activity and to potentially reduce the risk of urinary pooling. Obstructed menstruation rarely occurs. Some people believe that surgery in early childhood provides better outcomes and is less traumatic, although additional procedures or dilatation are often needed in adolescence, whereas others feel that surgery can be left until teenage years when the young person is able to be involved in the decision, is able to consent to the procedure and is able to engage in regular intercourse or dilatation. Most people believe that surgery can be deferred if there is a short common channel. Dilatation should not be performed in childhood.

Corporal (clitoral) reduction surgery in CAH is another debated area as this approach has been associated with decreased clitoral sensation in the past. The potential benefits of newer nerve-sparing techniques are under evaluation [40, 41]. Some people feel that the psychosocial benefits of early surgery include reduced parental anxiety, reduced potential 'stigmatization' for the child and avoiding clitoral surgery in adolescence, whereas others feel that clitoral surgery is a cosmetic procedure and it should be for the young person to decide if or when they have any surgery that affects their body autonomy and, potentially, sexual function. Optimal endocrine control is also very important. Significant reduction in the size of the clitoris can occur in the first 6 months of life once adrenal hyperandrogenism is under control, so parents should be counselled and supported in all options and should not view clitoral surgery as a 'fix it' approach. Similarly, poor endocrine control may be linked to clitoral growth or regrowth. These complex issues need ongoing input of an experienced team with psychological support. If surgical procedures are considered, they should only be undertaken by specialists with appropriate balanced information and informed consent. Surgery would nowadays rarely be considered for children with less than Prader 3 virilization and increasingly the child's ability to consent to irreversible surgery is being considered. Children with Prader 4 or 5 genitalia due to CAH are extremely challenging. Traditionally the presence of a uterus and ovaries and most likely female gender identity have led to most individuals with CAH being raised female.

Transition

Centres treating young people with CAH should have a defined referral pathway to adult care services. Ideally patients would attend dedicated transition clinics staffed

by adult and pediatric clinicians allowing a gradual transition to adult care usually around the end of secondary school at ~18 years of age.

Education and disclosure of medical information should occur in an age-appropriate manner and psychological counselling offered intermittently.

Guidelines recommend that females with CAH have a gynaecological history and examination under anaesthesia during adolescence but otherwise gynaecology examinations should be minimized.

Long-Term Outcome

Many studies have investigated the increasing cohort of adult patients with CAH examining a number of clinical and psychosocial health outcomes including medication regimen, final adult height, fertility, cardiovascular and metabolic risk profiles and quality of life.

Studies find increased rates of obesity, insulin insensitivity, elevated lipid concentrations and hypertension. Rates of osteopenia and osteoporosis approach 50%. Fertility and fecundity in women with CAH can be compromised by several factors such as polycystic ovaries, anovulation, previous surgeries and personal choices about having a family. In males, testicular adrenal rest tissue (TART) in the testis has been found in up to 69% of men investigated with ultrasound. These nodules can sometimes be palpable and correlate negatively with fertility. Overall quality of life in CAH was significantly impaired in both males and females in several studies [42].

The UK CaHASE study found that many patients had been lost to follow-up with poor transition strategies in place. Patients were mostly receiving non-physiological glucocorticoid replacement and androgen concentrations were poorly controlled. This study has signalled a need for improved transition of adolescent patients with CAH and improved clinical care in adults [43].

Prenatal Approaches to 21-OHD

In the past 25 years, considerable interest has focused on the prenatal diagnosis of 21-OHD and possible *in utero* treatments to try to reduce the genital changes in 46,XX children with this condition, when there is a known family history of 21-OHD and it is established that the parents are carriers. Treatments can be successful in their aim but remain controversial and experimental for several reasons.

During early fetal development, the HPA axis is intact and active from around 8 wpc. This period is also a critical time for genital development, so a 46,XX fetus with 21-OHD will be exposed to adrenal androgens and experience genital changes (clitoral growth, labial fusion, scrotalization) (Figure 9.21).

Although many steroids do not cross the placenta, dexamethasone administered to the mother does cross the placenta and can be used to suppress the HPA axis in the developing fetus. The steroid needs to be administered early in pregnancy to be effective (by 6–7 weeks) in relatively high doses (20 μg/kg maternal body weight/day or 1.5 mg for a 70 kg woman, i.e. supraphysiological doses) and is usually given for the duration of the pregnancy in affected babies.

The original experimental approach to prenatal treatment of 21-OHD was to administer dexamethasone early in pregnancy (before 6 weeks) if desired by parents who are known carriers of *CYP21A2* gene mutations. Amniocentesis or chorionic villus sampling was performed around 11–13 weeks to determine the fetal karyotype and treatment was stopped in 46,XY fetuses who would not benefit from it. As 21-OHD is a recessive condition, only one in four children are affected and only one in eight are affected 46,XX females. Therefore, seven of eight children would be exposed to high concentrations of dexamethasone during this critical early stage of development and three of four children with a 46,XX karyotype would be treated on an ongoing basis unnecessarily.

Several studies in animals and higher primates have shown that early exposure to dexamethasone *in utero* can have adverse neurodevelopmental effects, as well as effects on birth weight and metabolism but high doses were used and different models may respond differently. Human follow-up studies of children exposed to dexamethasone are less clear. Although there is no strong evidence for an excess of birth defects, there are limited data that show differences in short-term memory and some aspects of cognition in girls exposed to dexamethasone. These studies involved small numbers of children and have a limited length of follow-up but several professional societies recommend that prenatal use of dexamethasone is undertaken as an experimental protocol with full consent and the ability for long-term follow-up of all affected and unaffected children who have been treated.

In the past decade, several advances in molecular medicine have meant that potential treatments can be targeted more effectively. The ability to analyse cell-free fetal DNA (cffDNA) in maternal serum in pregnancy has been a breakthrough. Although concentrations of circulating fetal DNA are small, the amounts increase during pregnancy and analysis of Y chromosome markers has be used to identify whether the fetus is XY or XX from as early as 5 weeks' gestation in some centres. Using this approach, dexamethasone use in XY fetuses could be avoided, thereby preventing exposure of half of the fetuses unnecessarily.

Another advance has been to try to determine the fetal genotype in early pregnancy so that dexamethasone can be given only to *affected* XX fetuses. This approach is only useful if the parental genotypes (i.e. mutations) are

different and the paternal variant is a missense change that can be linked to markers in chromosome 6p21. Using this strategy, it has been possible to determine the presence of the mutant paternal allele in affected compared with unaffected fetuses and to target dexamethasone only to affected children. In such situations, the potential benefits and risks of treating an affected child with dexamethasone can be considered on an individual basis [44, 45].

11β-Hydroxylase (CYP11B1) Insufficiency

11β-Hydroxylase (encoded by CYP11B1) mediates the 11β-hydroxylation of 11-deoxycortisol to cortisol and that of DOC to corticosterone in the *zona fasciculata* and *zona glomerulosa* (Figure 9.6). Defects in P450c11β result in a block in cortisol synthesis, reduced negative feedback and a rise in ACTH that result in a build-up of precursors and shunting of steroids into the androgen pathway.

Children with P450c11β deficiency have decreased cortisol secretion and virilization. The clinical effects of the cortisol deficiency can be masked by the glucocorticoid activity of high concentrations of corticosterone, so severe AI is uncommon. High concentrations of DOC cause sodium retention with time, resulting in hypokalaemia, hypernatraemia and hypertension, but a mild transient salt loss can occur in the days after birth due to relative mineralocorticoid insufficiency and before the high molar concentrations of DOC have occurred. This feature, together with elevated 17-OHP, can lead to a misdiagnosis of 21-OHD.

The virilization associated with P450c11β deficiency can be severe, resulting sometimes in Prader 4 or 5 genitalia. Children with a 46,XX karyotype may be assumed to be boys with non-palpable testes but will not manifest the severe salt-losing crisis seen in 21-OHD. Rather, children present later with hypertension and both 46,XX and 46,XY children can develop gonadotropin-dependent precocious puberty due to adrenal hyperandrogenism in early childhood. Milder forms of P450c11β deficiency have also been reported in women with hyperandrogenism and menstrual irregularities.

The diagnosis of P450c11β deficiency can be made biochemically by elevated ACTH, an attenuated cortisol response to Synacthen stimulation, elevated DOC and 11-deoxycortisol, low aldosterone and normal/suppressed renin despite hypertension and hypokalaemia. The USP has characteristic patterns or metabolites and genetic analysis of *CYP11B1* can confirm the diagnosis.

P450c11β deficiency accounts for ~5% of cases of CAH in Europe but it is an important diagnosis to make as patients can be mislabelled as having 21-OHD. P450c11β deficiency is more common in Middle Eastern populations especially in Sephardic Jews of Moroccan ancestry (p.Arg448His) and in areas of Turkey.

Treatment is with glucocorticoids to suppress ACTH drive. Adjuvant treatment of hypertension and hyperandrogenism may be required. GnRH agonists may be needed to prevent the development of central precocious puberty once the peripheral androgens have been suppressed.

Adrenal Excess

Adrenal hormone excess is a feature of many adrenal diseases and can include glucocorticoid, mineralocorticoid or androgen excess in isolation or combined (Table 9.7). The clinical features depend on the steroids involved and the rate of onset of the condition.

Any form of glucocorticoid excess is known as Cushing syndrome, whereas the ACTH-dependent form due to a pituitary adenoma is called Cushing disease.

Mineralocorticoid excess is rare in children but includes aldosterone-producing adenomas (Conn syndrome), familial and primary hyperaldosteronism and AME.

Excess levels of adrenal androgen precursors DHEA and androstenedione can be due to many different causes ranging from adrenal tumours to idiopathic premature adrenarche. Gonadal causes of androgen excess also need to be considered as an important part of the differential diagnosis (Table 9.7; see also Chapter 4).

Glucocorticoid Excess (Cushing Syndrome)

CS can be classified into adrenocorticotrophic hormone (ACTH)-dependent and ACTH-independent causes (Table 9.7). Cushing disease, which is caused by an ACTH-secreting pituitary corticotroph adenoma, is the commonest cause of CS accounting for 75–80% of pediatric CS cases compared with 49–71% of adult cases. CS caused by ACTH of non-pituitary origin is termed the ectopic ACTH syndrome.

ACTH-independent causes of CS include adrenocortical tumours (ACTs) (e.g. adrenal carcinomas or adenomas), primary adrenal hyperplasia (e.g. primary pigmented nodular adrenocortical disease [PPNAD]) and macronodular adrenal hyperplasia (e.g. ACTH-independent macronodular adrenal hyperplasia [AIMAH], McCune–Albright syndrome [MAS]). Many pediatric adrenal tumours secrete adrenal androgens as well as glucocorticoids so presentation with predominant features of glucocorticoid excess is relatively rare.

Iatrogenic exogenous glucocorticoid administration remains the most common cause of CS and the source of the steroids may not be immediately apparent without direct questioning (e.g. dexamethasone eye drops).

Table 9.7 Causes of glucocorticoid, mineralocorticoid and adrenal hormone excess in childhood, including gonadal causes of androgen excess.

Glucocorticoid excess

ACTH dependent
1) Cushing disease (ACTH-secreting pituitary adenoma)
2) Ectopic ACTH syndrome

ACTH independent

1) Exogenous glucocorticoid administration (tablets, inhalers, nose drops, nasal spray, skin cream)
2) Adrenocortical tumour producing glucocorticoid (adenoma or carcinoma)
3) Primary adrenocortical hyperplasia
   i) Micronodular disease
      a) Isolated primary pigmented nodular adrenocortical disease (PPNAD)
      b) PPNAD with Carney complex
      c) Isolated micronodular adrenocortical disease (no or minimal pigmentation)
   ii) Macronodular adrenal hyperplasia
      a) Bilateral macroadenomatous hyperplasia (BMAH)
      b) McCune–Albright syndrome
      c) ACTH-independent macronodular adrenal hyperplasia (AIMAH)

Mineralocorticoid excess

1) Conn syndrome (aldosterone-producing adenoma)
2) Bilateral adrenal hyperplasia
   a) Familial hyperaldosteronism type 1, FHA 1
   b) Familial hyperaldosteronism type 2, FHA 2
   c) Familial hyperaldosteronism type 3, FHA 3
   d) Primary idiopathic hyperaldosteronism
3) Apparent mineralocorticoid excess (11β-hydroxysteroid dehydrogenase type II deficiency)
4) Mineralocorticoid receptor activation

Androgen excess

High/pubertal range gonadotropins

1) Central precocious puberty
   a) Idiopathic precocious puberty
   b) Central nervous system lesions

Low/prepubertal gonadotropins
1) Gonadal androgen production
   a) Familial testotoxicosis
   b) βHCG producing tumours
   c) Gonadal tumour producing androgens
2) Adrenal androgen production
   a) Adrenal tumour producing androgens (adenoma or carcinoma)
   b) Congenital adrenal hyperplasia
   c) Cortisone reductase deficiency (11β-hydroxysteroid dehydrogenase type I deficiency)
   d) Apparent cortisone reductase deficiency (hexose-6-phosphate dehydrogenase deficiency)
   e) Apparent DHEA sulphotransferase deficiency (PAPS synthase 2 deficiency)
   f) Idiopathic premature adrenarche

Epidemiology

Excluding iatrogenic causes, CS is rare in children with an overall incidence of 2–5 new cases per million children per year. Although the age of onset can vary, Cushing disease is the commonest cause in school-age children with a peak incidence around 14.1 years. Adrenal tumours predominate in infants and preschool children. Infantile CS is a feature of MAS.

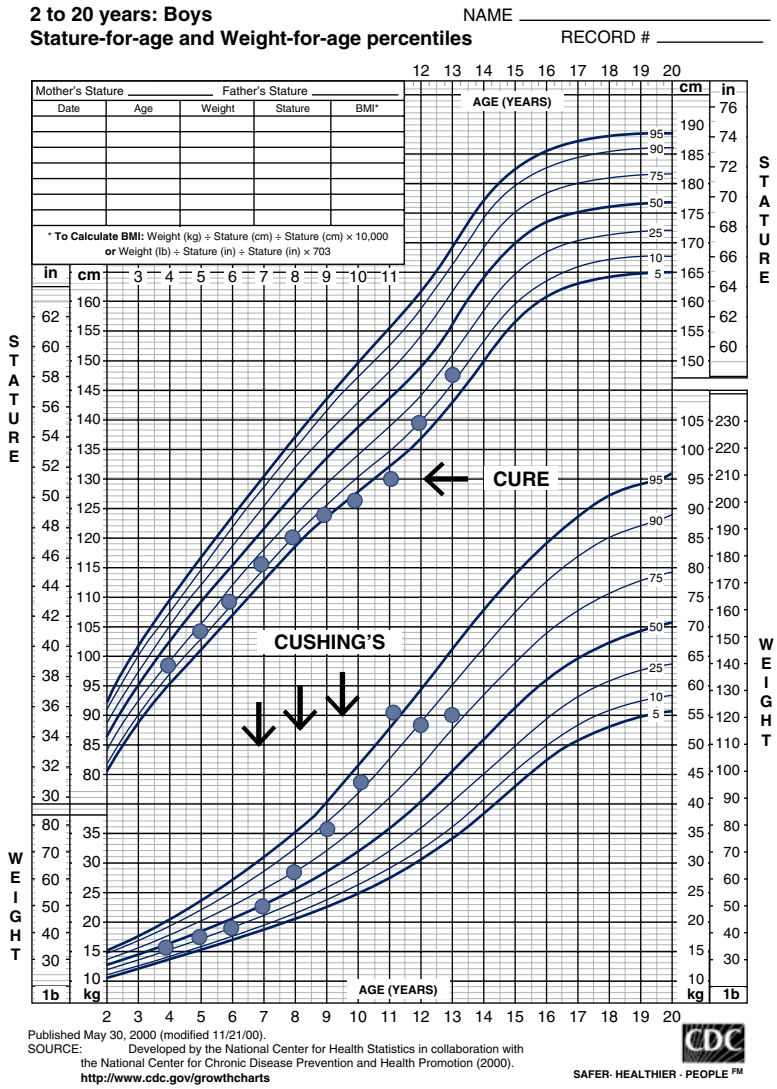
Cushing disease has a female preponderance in adults but the sex ratio is equal in adolescents and there is a higher proportion of affected males in prepubertal patients. The explanation for this is unclear.

Clinical Features

Two of the earliest and most reliable features of CS are growth failure and weight gain (Figure 9.25, Table 9.8). Children and adolescents may have a typical Cushingoid appearance with 'moon facies'. Additional features include hirsutism, acne, facial plethora and striae. Virilization with apparent precocious puberty can be an important presenting feature, although pubertal delay or arrest can also occur. Psychological disturbances (including emotional lability/depression), hypertension, headaches and fatigue are noted in some children. Muscle weakness and easy bruising are rare. Hyperlipidaemia and elevated blood glucose concentrations, including insulin-resistant diabetes, can be found. The obesity of CS in children is initially generalized rather than centripetal and a buffalo hump is evidence of long-standing disease. Cyclical features have been reported.

The change in the child's appearance occurs over time and is often not appreciated until after the diagnosis is made and previous photographs are studied. A significant delay in diagnosis following onset of symptoms is

(a)



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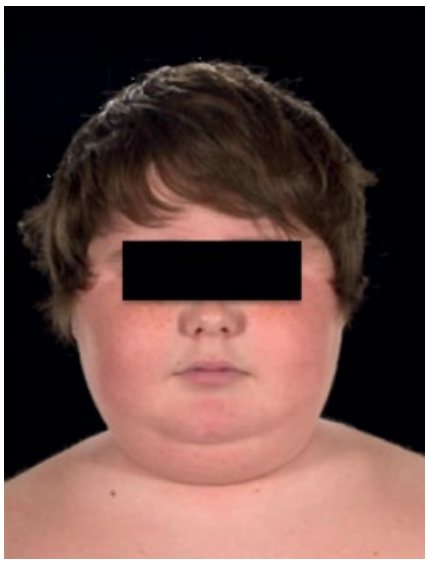


Figure 9.25 Clinical features of childhood Cushing syndrome. (a) Typical growth chart for a boy with Cushing syndrome (Cushing disease). (b) Typical facial features of Cushing syndrome (moon facies, plethora). *Source:* Courtesy of Professor Mehul Dattani.

Table 9.8 Clinical features of Cushing disease in children and young people compared with adults.

Feature	Pediatric Cushing disease, % (*n* = 41) [46]	Pediatric Cushing disease, % (*n* = 39) [47]	Adult Cushing disease, % (*n* = 183) [46]	Mean age of onset in pediatric Cushing disease, age ± S.D. [46]
Facial changes	100	—	81	12.3 ± 3.5
Weight gain	98	92	65	12.3 ± 3.5
Virilization	76	—	22	10.5 ± 2.8
Growth failure	—	84	—	—
Delayed/arrested puberty	—	60	—	—
Fatigue	61	67	26	11.6 ± 3.6
Hirsutism	59	46	68	12.6 ± 3.3
Emotional lability/ depression	59	—	41	11.8 ± 3.1
Headaches	51	26	31	12.7 ± 3.2
Striae	49		40	14.2 ± 2.6
Hypertension	49	63	77	11.8 ± 3.5
Acne	44	46	27	13.9 ± 2.2
Compulsive behaviour	—	44	—	—
Striae	—	36	—	—
Bruising	—	28	—	—
Buffalo hump	—	28	—	—

Other features include osteopenia, nocturia and impaired glucose tolerance.

frequent, the mean length of symptoms prior to diagnosis in a series of 37 patients with Cushing disease being 0.3–6.6 years (mean 2.5 years). CS caused by adrenal carcinoma or the ectopic ACTH syndrome can produce a rapid fulminant course.

Growth failure has been attributed to GHD, insulin-like growth factor 1 (IGF-1) resistance and prolonged high concentrations of circulating glucocorticoids. Obesity is extremely common but there is a crucial difference in the growth pattern between obesity due to CS and so-called 'simple' obesity; simple obesity causes an increase in growth velocity, while CS is almost always associated with growth failure (Figure 9.25).

Investigation of Cushing Syndrome

Investigations in children suspected of having CS are largely based on those performed in adults. Initial investigations aim to confirm or exclude the diagnosis and then aim to determine the aetiology. A recent consensus statement advised that only obese children who have demonstrated a reduction in growth velocity should be investigated.

Confirmatory investigations include an initial screening test of 2–3 consecutive 24 hour urine collections for UFC. This measures only free, unbound hormone and is not affected by conditions or medications that alter cortisol-binding globulin (CBG). Measurement of serum cortisol at 3 time points (0900 and 1800 hours and midnight [sleeping]) can be used to assess circadian rhythm. Determination of midnight cortisol in the sleeping child gives the highest sensitivity for the diagnosis of CS, as the concentration in unaffected children is usually <50 nmol/L although some young children may reach their cortisol nadir earlier than midnight. Patients may need to be admitted overnight to obtain serum cortisol concentrations. Salivary cortisol concentrations measured at different time points can also be used.

Originally described by Liddle in 1960, the dexamethasone suppression test involves administration of low doses of dexamethasone, a potent synthetic glucocorticoid that suppresses secretion of pituitary ACTH and cortisol in normal patients. Low doses of dexamethasone fail to suppress cortisol concentrations in endogenous CS of any cause. As dexamethasone also suppresses adrenal androgen secretion, this test is useful for distinguishing between adrenal and gonadal sources of sex steroids.

A dexamethasone suppression test requires the measurement of basal values and those obtained in response to either low- or high-dose dexamethasone but there is no benefit in administering the high-dose dexamethasone test to patients with Cushing disease who showed

reduction of cortisol during the low-dose test. The usefulness of the high-dose dexamethasone suppression test in children is questionable.

The low-dose dexamethasone suppression test (LDDST) uses the adult dose regimen of 0.5 mg 6 hourly (at 0900, 1500, 2100 and 0300 hours) for 48 hours or 30 μg/kg/day if the child weighs <40 kg. Blood is taken for serum cortisol at 0 and at 48 hours, 6 hours after the last dose, when it is normally undetectable (<50 nmol/L). There is debate over the ideal cut-off to maximize the specificity and sensitivity of this test. Because some patients with Cushing disease demonstrate some degree of suppressibility, the lower cut-off of <50 nmol/L rather than, for example, 140 nmol/L, improves sensitivity with rates approaching 95%.

Following confirmation of CS, investigations are required to determine its cause. Determination of basal plasma ACTH confirms Cushing disease. In ACTH-independent CS, ACTH should be undetectable. The CRH test (1 μg/kg IV) is of questionable use in children as ectopic ACTH syndrome is so rare but an increased cortisol response following CRH stimulation supports a diagnosis of Cushing disease rather than ectopic ACTH syndrome.

Cushing Disease

Pediatric Cushing disease is almost always caused by a pituitary microadenoma with diameter <5 mm. Pituitary macroadenomas have been described as an early manifestation of multiple endocrine neoplasia type 1 (MEN1) in children and therefore should raise the possibility of this diagnosis. Somatic mutations in USP8 have also recently been reported in ~30% of pediatric Cushing disease.

Most pediatric endocrinologists have limited experience in the diagnosis and treatment of children with Cushing disease and may benefit from close consultation with adult colleagues but there are some notable differences between pediatric and adult Cushing disease in terms of clinical features as well as investigations (Table 9.8). In contrast to adults there is an increased frequency in prepubertal males compared with females. In children, although there is frequently no corticotroph adenoma on pituitary scanning, there is a higher incidence of lateralization of ACTH secretion with inferior petrosal sinus sampling. Children also have a more exuberant cortisol response to IV CRH and a more rapid response to external beam pituitary radiotherapy compared with adults.

Pituitary imaging using MRI identified a microadenoma in ~50% of pediatric patients in a large NIH series. The majority of microadenomas have a hypointense signal on MRI, which fails to enhance with gadolinium. In some cases it may be difficult to differentiate between Cushing disease and ectopic ACTH syndrome. In these cases a CT scan of the chest using 0.5 cm cuts can be helpful to exclude a bronchial carcinoid tumour but this is very rare in young people.

Bilateral inferior petrosal sinus sampling (BIPSS) for ACTH is a highly specialized technique used in adults and children to help localize the microadenoma by identifying lateral or midline ACTH secretion. BIPSS can better predict the site of the microadenoma than pituitary imaging.

Treatment options have significantly advanced over the last 50 years. In the past bilateral adrenalectomy was treatment of choice and, while effective in curing hypercortisolaemia, patients required lifelong glucocorticoid and mineralocorticoid replacement. In addition there was a small but appreciable risk of post-adrenalectomy Nelson syndrome. Medical therapy to lower cortisol using metyrapone, ketoconazole or etomidate is useful for short-term therapy before surgery or radiotherapy but not as long-term therapy.

The definitive cure of Cushing disease can be achieved by trans-sphenoidal pituitary surgery (TSS) or radiotherapy. TSS is now considered first-line therapy as it involves removal of the adenoma while maintaining normal pituitary tissue *in situ*. This approach is generally safe and effective in children and low rates of post-operative hypopituitarism have been reported. Selective microadenomectomy can be technically very challenging in children due to their small size and careful post-operative management of steroid weaning and water balance is needed. Pituitary radiotherapy is also safe and effective and is often used as a second-line therapy if TSS is unsuccessful. It carries risks of affecting other pituitary hormones with time.

Prognosis for cure after TSS is good in most children and adolescents with full recovery of the HPA axis but post-treatment management frequently presents challenges for optimization of growth, puberty and body composition. Children often remain obese and several studies have reported a poor outcome for post-treatment catch-up growth and adult height. As there is a finite time available for normal growth, it could be argued that rapid diagnosis and treatment is even more important in children than in adults [48–51].

Ectopic ACTH Syndrome

Ectopic ACTH-producing tumours are extremely rare in childhood and their prevalence is <1% of all adolescents with CS. Co-secreting ACTH/CRH tumours have been reported. One large multicentre national study identified 10 cases of pediatric ectopic ACTH syndrome over a 23-year period; all patients were over 14 years of age at presentation. In this series, 8 of the 10 tumours were neuroendocrine tumours (mostly well-differentiated

endocrine lung tumours), 1 was Ewing's sarcoma, and 1 was a nested stromal epithelial tumour of the liver. Analysis of the literature reveals that the aetiology of pediatric ectopic ACTH syndrome differs with age. In children <4 years, most are embryonic tumours. After 8 years of age, some unusual aetiologies are described but bronchial, thymic and pancreatic neuroectodermal tumours account for most cases, similar to many adult series.

Ectopically produced POMC and ACTH are derived from the same gene that produces pituitary POMC but it is not sensitive to glucocorticoid feedback in the malignant cells. This phenomenon permits distinction between pituitary and ectopic ACTH as pituitary-dependent ACTH secretion is suppressed by high doses of dexamethasone, whereas ectopic ACTH syndrome is not suppressed and typically associated with ACTH concentrations 10–100 times higher than those seen in Cushing disease. This rare diagnosis should be considered in children with ACTH-dependent CS with no unequivocal pituitary lesion at MRI. Clinical features of CS are often not so obvious as the time course is more rapid than in other forms of glucocorticoid excess. Hypokalaemic alkalosis and hypertension are more frequently seen as extremely high concentrations of ACTH can stimulate aldosterone synthesis and secretion. CT of the neck and chest should be the primary imaging study to localize ectopic ACTH syndrome tumours.

The optimal treatment of tumours leading to ectopic ACTH syndrome is surgical excision. Control of excessive cortisol concentrations can be achieved with inhibitors of steroidogenesis while waiting to localize the tumour and prepare for surgical removal, minimizing the need for bilateral adrenalectomy.

Adrenal Tumours

ACTs are rare in children and adolescents with an estimated annual incidence of 0.2–0.3 cases per million in the USA; internationally, the incidence of ACT varies substantially and is particularly high in southern Brazil, ~10–15 times that of observed in the USA due to a familial genetic predisposition. ACTs follow a bimodal age distribution with peaks during the first and fourth decades and most studies reporting a median age of 3 years in children. In children there is a predominance of girls with reported ratios of 1.6 : 1 but this varies widely among age groups.

Ninety percent of ACTs are associated with clinical features of hormone excess. Only 10% of ACTs are non-functioning tumours. The most common presenting sign is virilization in 80–90% of patients. Thirty percent of patients have concomitant signs of glucocorticoid excess and, rarely, increases in aldosterone or oestrogen. Virilizing features are especially common in young children. They include acne, clitoromegaly, precocious puberty, voice changes and hirsutism. Adolescents and young adults are more likely to present with CS or non-functional tumours with an abdominal mass. Hypertension is frequently observed at the time of diagnosis and usually attributed to tumour production of either glucocorticoids (in most cases) or mineralocorticoids. Hypertension can also occur in patients with virilizing or non-functional tumours due to tumour compression of the renal artery.

Children with suspected or confirmed ACT should be managed by a designated professional multidisciplinary team (MDT) led by an age-appropriate endocrinologist, with experience in the management of ACT.

The diagnosis requires confirmation of excess adrenocortical steroid secretion in the case of functioning ACTs or the exclusion of other adrenal tumours (e.g. phaeochromocytoma, paraganglioma, neuroblastoma) for non-functioning ACTs. All children with suspected ACT should have baseline investigations that include U&E and bone profile, DHEA-S, androstenedione, 17-OHP, 11-DOC, testosterone and oestradiol. All children should have investigations to exclude excess cortisol excretion as this may not be clinically evident but has important implications for steroid requirements during and after surgery. Renin and aldosterone should be measured, especially if a patient demonstrates hypertension or hypokalaemia. It may be necessary to exclude CAH and premature adrenarche.

A 24 hour urinary steroid profile can provide additional information regarding tumour hormone secretion and potentially a tumour marker for future disease monitoring. Radiological investigations initially include abdominal ultrasound followed by MRI.

Pediatric ACTs can be categorized into benign adrenocortical adenoma, intermediate for malignancy or of uncertain malignant potential and adrenocortical carcinoma (ACC). It is often difficult to characterize pediatric ACTs as they behave differently from ACTs in adults and the well-established Weiss criteria used in adults often over-predicts the diagnosis of malignancy in pediatric ACTs, with significant implications for adjuvant therapy and follow-up. It is not possible to characterize ACTs as benign or malignant radiologically unless local invasion or distant metastases are present.

All patients diagnosed with ACTs should be referred to a cancer genetic service as ~10% of all patients with ACTs will have an identifiable hereditary syndrome that can be diagnosed by genetic testing. Most patients, including ~50% of children with very-early-onset ACC, have germline *TP53* mutations and the associated Li–Fraumeni syndrome. Some patients may not have the classical criteria required to diagnose Li–Fraumeni

syndrome probably due to significant variability in preserved TP53 function with different mutations.

Patients from southern Brazil have a well-described hotspot mutation in TP53 (p.Arg337His) that accounts for the increased predisposition in this population. Other hereditary syndromes associated with ACCs include Beckwith–Wiedemann syndrome, Lynch syndrome, MEN1 and, rarely, familial adenomatous polyposis.

There is no indication to biopsy adrenal tumours in children as this is likely to cause tumour seeding and recurrence. Surgery is the single most important procedure in the successful treatment of ACT. Complete resection is required for cure; adjuvant therapy remains controversial and is usually ineffective in patients with microscopic or gross residual disease.

There is no consensus on disease staging of childhood ACTs. Available classifications are based on tumour size, lymph node involvement, complete resection and metastatic disease. Tumour size is often reported as the most significant prognostic factor as patients with completely resected small tumours (<200 g) have an excellent prognosis. In a large multivariate analysis, disease stage, presenting signs of endocrine dysfunction and age were independently associated with prognosis. The 5-year event-free survival estimate is ~50% [52].

Primary Adrenocortical Hyperplasia

ACTH-independent multinodular adrenal hyperplasia is rare and bilateral and comprises macronodular and micronodular disease. Micronodular disease, including PPNAD and Carney complex (CNC), usually has a genetic aetiology but macronodular bilateral adrenocortical hyperplasia is less frequently genetic and more commonly presents in later life, with the exception of MAS. Adrenalectomy is usually required to treat these cases although in some circumstances subtotal resections may be indicated.

Micronodular Adrenal Disease (Carney Complex, Isolated Primary Pigmented Nodular Adrenocortical Disease [PPNAD] and Micronodular Adrenal Hyperplasia)

Micronodular adrenal disease describes multiple microadenomatous lesions, usually <1 cm. Microadenomatous hyperplasia can occur with pigment (lipofuscin) and usually some intranodular atrophy and is termed primary pigmented nodular adrenocortical disease. This can occur in isolation and is associated with changes in *PRKAR1A* (PPNAD1) as well as in the phosphodiesterase genes *PDE11A* (PPNAD2) and *PDE8B* (PPNAD3).

PPNAD is the most frequent endocrine manifestation of CNC, which is an autosomal dominant multiple neoplasia syndrome most commonly linked with inactivating mutations in *PRKAR1A*. Other features of CNC include spotty skin pigmentation and lentigines, cardiac myxomas, endocrine overactivity and schwannomas.

Isolated micronodular adrenocortical disease can be associated with phosphodiesterase 11A and phosphodiesterase 8B defects as well as other genomic loci including 2p12–p16 and 5q.

Macronodular Adrenal Disease (Bilateral Macroadenomatous Hyperplasia [BMAH], McCune–Albright Syndrome, ACTH-Independent Macronodular Adrenal Hyperplasia)

Macronodular hyperplasias comprise multiple nodules more than 1cm diameter. Usually two or three distinct adenomas are detected with intranodular atrophy, termed bilateral macroadenomatous hyperplasia (BMAH). These usually occur in middle age and can result from somatic activating mutations in GNAS (Gs alpha subunit).

Systemic mosaicism for somatic activating mutations in GNAS causes MAS. In contrast to BMAH, MAS usually presents in infants or young children and can present with signs and symptoms of florid CS. Other features of MAS include polyostotic fibrous dysplasia, hyperpigmented skin lesions, endocrine overactivity (e.g. hyperthyroidism) and early menstruation in girls.

AIMAH is distinguished by multiple adenomatous hyperplasia but with intranodular *hyperplasia* of the *zona fasciculata*. This is associated with Gs alpha-subunit activation in the adrenal glands or 'ectopic' receptor expression and is more prevalent in adulthood.

Mineralocorticoid Excess

Mineralocorticoid excess is rare in children. The two most common causes in adults are both characterized by constitutive overproduction of aldosterone by aldosterone-producing adenomas and bilateral adrenal hyperplasia (BAH). BAH can include genetic syndromes such as familial hyperaldosteronism and primary idiopathic hyperaldosteronism. The latter describes patients with hypertension, high aldosterone concentrations and low PRA with no evidence of an adenoma or familial hyperaldosteronism.

Conn Syndrome

Conn syndrome is caused by an aldosterone-producing adenoma and is extremely rare in children. It is characterized by hypertension, polyuria and muscular weakness. Biochemical investigations show a hypokalaemic metabolic alkalosis with high aldosterone concentrations and low PRA. Imaging and adrenal vein sampling may be needed to distinguish unilateral and bilateral adrenal aldosterone production. Usually the cause is a small adrenal adenoma confined to the *zona glomerulosa* of one adrenal, which can be cured by surgical resection.

Bilateral Adrenal Hyperplasia

Glucocorticoid Suppressible Hypertension (Glucocorticoid Remediable Aldosteronism [GRA], Familial Hyperaldosteronism Type I [FHA1])

Patients with glucocorticoid suppressible hypertension (familial hyperaldosteronism type I [FHA1]) present with hypertension, often in childhood, although a mild clinical phenotype has been described. Biochemical investigations demonstrate high aldosterone concentrations, low PRA and high urinary 18-hydroxycortisol (18-OHF). Potassium can be low or normal.

This is an autosomal dominant condition caused by a chimeric *CYP11B1*/*CYP11B2* gene. Consequently, aldosterone synthase is expressed in the *zona fasciculata* and aldosterone production is under the regulation of an ACTH-sensitive gene promoter instead of angiotensin II. As a result, aldosterone production follows the same circadian secretion pattern as cortisol.

Treatment is with glucocorticoids to suppress ACTH and therefore aldosterone secretion. Only partial suppression of ACTH is required for correction of hypertension. MR antagonists can also be used if needed.

Familial Hyperaldosteronism Type II (FHA2)

Patients are diagnosed with familial hyperaldosteronism type II (FHA2) when at least two first-degree relatives have confirmed primary hyperaldosteronism but FHA1 and familial hyperaldosteronism type 3 (FHA3) have been excluded. Clinical and biochemical features vary, possibly reflecting genetic heterogeneity, but some families have been linked to a chromosome 7p22 locus.

KCNJ5 Deficiency (Familial Hyperaldosteronism Type 3 [FHA3])

FHA3 is characterized by severe resistant hypertension of childhood onset with significant BAH. On histopathology there is an atrophic *zona glomerulosa* but diffuse hyperplasia of the *zona fasciculata*. Biochemistry reveals high aldosterone, low PRA and marked hypokalaemia with high urinary concentrations of 18-OHF and 18-oxocortisol.

FHA 3 is due to dominant mutations in *KCNJ5*, which encodes the potassium ion channel Kir3.4. Expression of mutant *KCNJ5* leads to higher membrane depolarization and calcium influx through voltage-gated calcium channels. This results in increased aldosterone release and cell proliferation.

Treatment is challenging as paradoxically there is a marked increase in aldosterone and blood pressure in response to dexamethasone. Patients may require adrenalectomy.

11β-Hydroxysteroid Dehydrogenase Type II Deficiency (Apparent Mineralocorticoid Excess [AME])

AME is primarily a renal disease with adrenal consequences. Recessive mutations in *11β-HSD2* result in decreased conversion of active cortisol to inactive cortisone in the kidney (Figure 9.9; see '11β-hydroxysteroid dehydrogenase' section). Increased cortisol availability results in inappropriate activation of the MR by cortisol leading to hypertension but with low aldosterone concentrations and low PRA. GC-MS of urinary steroids shows increased ratio of cortisol (THF + 5α-THF) to cortisone (THE) metabolites. Liquorice contains glycyrrhetinic acid, which inhibits renal 11β-HSD2 activity and can result in transient AME.

Mineralocorticoid Receptor Activation

One family has been reported with early-onset hypertension and pregnancy-induced hypertension due to a dominantly inherited p.Ser810Leu gain-of-function mutation in the MR (NR3C2). This variant causes mild constitutive activation and inappropriate responsiveness to progesterone, accounting for the exacerbation during pregnancy.

Androgen Excess

Excess adrenal androgen precursors DHEA and androstenedione lead to symptoms and signs of virilization. It is imperative to determine the exact aetiology; similar clinical features can be associated with central or peripheral precocious puberty, CAH or adrenal tumours.

If these diagnoses have been excluded and isolated adrenal androgen excess confirmed, the child might have one of several single-gene disorders but, even collectively, these are very rare.

Idiopathic premature adrenarche (exaggerated adrenarche) is by far the most common cause of adrenal androgen excess in late childhood, once non-classic CAH is excluded. This condition is poorly understood and is effectively a diagnosis of exclusion.

11β-Hydroxysteroid Dehydrogenase Type I Deficiency (Cortisone Reductase Deficiency [CRD])

11β-HSD1 interconverts cortisone and cortisol via its oxo-reductase and dehydrogenase activities but 11β-HSD1 oxo-reductase activity predominates *in vivo* causing activation of cortisone to cortisol (Figure 9.9, '11β-hydroxysteroid dehydrogenase' section). Heterozygous mutations in 11β-HSD1 cause decreased conversion of inactive cortisone to active cortisol. This leads to loss of cortisol regeneration and increased cortisol clearance, resulting in secondary activation of the HPA axis and subsequent adrenal androgen excess. GC-MS of urinary steroids shows increased ratio of cortisone (THE) to cortisol (THF + 5α-THF) metabolites and elevated androgenic precursor androsterone and etiocholanolone.

Hexose-6-Phosphate Dehydrogenase Deficiency (Apparent Cortisone Reductase Deficiency [ACRD])

H6PD generates the cofactor NADPH in the endoplasmic reticulum that is used by 11β-HSD1 for the conversion of cortisone to cortisol (Figure 9.6; see '11β-hydroxysteroid dehydrogenase' section). Recessive mutations in H6PD lead to a similar biochemical profile to CRD but more marked changes in the urinary steroid profile. In addition to presenting with androgen excess in childhood, patients may also present in adulthood with PCOS.

PAPS Synthase 2 Deficiency (Apparent DHEA Sulphotransferase Deficiency)

DHEA can either be converted to the active androgens, testosterone and 5α-dihydrotestosterone, allowing activation of the AR, or alternatively can undergo sulphation and be converted to inactive DHEA-S via DHEA sulphotransferase (SULT2A1) (Figure 9.8; see 'Steroid sulphotransferase and sulphatase' section). PAPSS2 provides adrenal SULT2A1 with its sulphate donor and so is essential for converting DHEA to DHEA-S. Loss-of-function mutations in PAPSS2 therefore result in a decrease in the pool of inactive sulphated DHEA-S and importantly an excess of precursor available for conversion to active androgens. Mutations in *PAPSS2* have been reported in patients with hyperandrogenism and PCOS. In more severe cases, skeletal abnormalities (brachyolmia type 4 with epiphyseal and metaphyseal changes) and short stature have also been reported indicating a multisystem disorder. Biochemically patients demonstrate low concentrations of DHEA-S with normal or high DHEA and high concentrations of androstenedione and testosterone. Although rare, these cases have identified DHEA sulphation as a regulator of androgen synthesis (Figure 9.8).

Idiopathic Premature Adrenarche

Adrenarche refers to the developmental maturation of the *zona reticularis* and the resultant increase in adrenal androgen precursor DHEA and its sulphate ester, DHEA-S (see 'Adrenal androgen production and the regulation of adrenarche' section). This phenomenon occurs in humans, gorillas and chimpanzees and its regulation and the exact control of adrenal androgen secretion remain to be elucidated. Adrenarche is thought to be a continuous developmental process with increasing concentrations of DHEA detected from as early as 3 years of age but with concentrations rising significantly from about 6 years in girls and 8 years in boys. Significant changes in the activity of certain enzymes within the steroidogenic pathway occur; an increase in 17,20-lyase activity and DHEA sulphotransferase (SULT2A1) with reduced HSD3B2 activity lead to a change in the pattern of steroid secretion in response to ACTH stimulation and ultimately increased DHEA and androstenedione concentrations.

Adrenarche is usually manifested by pubic and axillary hair development, adult-type body odour and oily skin with no signs of pubertal development. There is often an associated modest increase in growth rate but this has no major impact on final height.

Premature (or exaggerated) adrenarche is defined by increased concentrations of DHEA and DHEA-S with associated clinical features before the age of 8 in girls or 9 in boys. It is more common in children from certain backgrounds and was considered an extreme variant of normal with no medical consequences providing all pathological causes had been excluded. Bone age may be advanced and this may be associated with a reduced final height. Recent research suggests that children with premature adrenarche may be at increased risk of weight gain, PCOS and metabolic syndrome in later life. Some groups have suggested that children born with low birth weight are at increased risk of premature adrenarche but this has not been supported by further research in children with diverse ethnic backgrounds [11, 12].

Education, Support and Long-Term Care

Education

AI disorders in children are rare but potentially fatal if untreated. Parents and other adults (e.g. school teachers) who take care of an affected child must be equipped with the knowledge and capability to manage the child's condition daily and during an emergency. Providing key information and support during the initial days is particularly important for new parents and families whose child has been diagnosed with AI because they may often feel anxious. Understanding new information can be difficult while coming to terms with the diagnosis.

Key information for parents/carers should include:

- Basic facts about the condition and how it is managed.
- Treatment being simple, effective and part of a lifelong daily routine for a child on steroid replacement therapy. With a right balance of steroid replacement, the child can live a normal life like their peers.
- Medications and the common side effects.
- Written action plan and/or flow chart with information on how to deal with acute situations such as illnesses, accidents and other stressful events (e.g. trauma, surgery); the 'sick day rules'.
- Symptoms of an adrenal crisis in a child and how to recognize them.

- Information and illustrations on how and when to give an emergency injection of hydrocortisone in the event of an adrenal crisis.
- Details of the primary medical care team and regional emergency contact.
- Important life-saving items to be carried by the child and kept at school for school-age children always:
  - Engraved medical identity bracelet or necklace with child's condition, emergency treatment and contacts.
  - Medical alert card with the child's treatment details and emergency contacts.
  - Emergency kits with user instruction leaflets (including glucose gel).

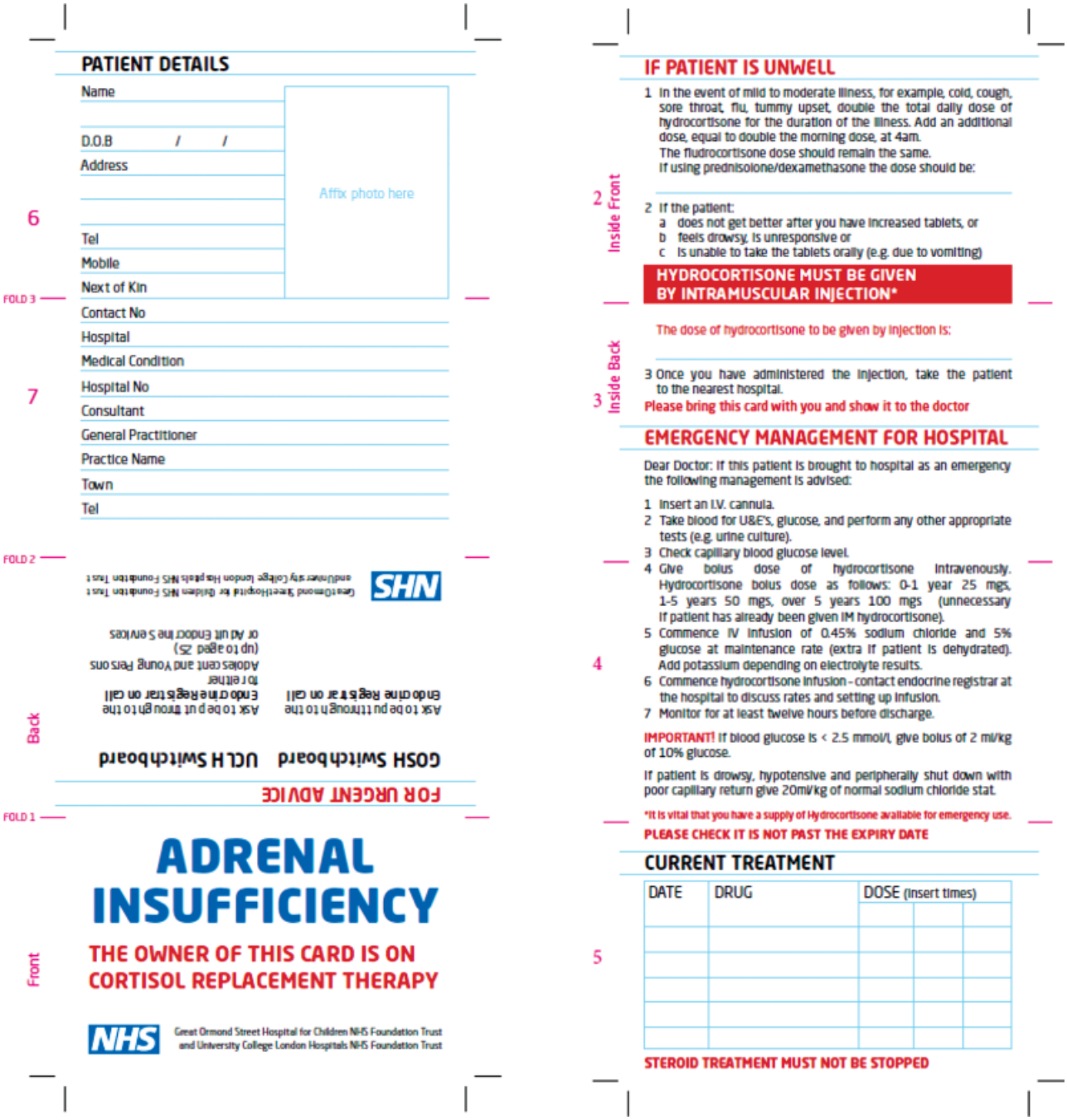


Figure 9.26 Example of a medical alert card for individuals with adrenal insufficiency. *Source:* Modified with permission from resources produced by Great Ormond Street Hospital for Children NHS Foundation Trust (2015).

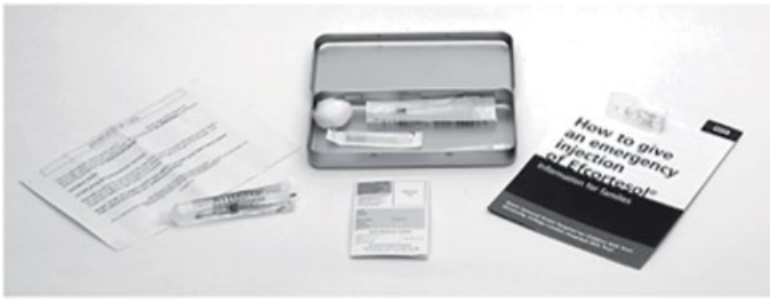
Some examples of medical information, emergency kits and management flow charts for caregivers are shown in Figures 9.26–9.28. Information sheets should be written in simple, non-technical languages suitable for local use. Technical medical terms within the context should be fully explained in the medical glossary. Pictorial illustrations and visual aids (e.g. videos or smartphone apps) should be ready for use. Parents/carers of specific patient groups should also be aware of special medical considerations such as patients on DDAVP for coexisting diabetes insipidus during an adrenal crisis, non-compliance with treatment (especially in adolescents), travel advice and emergencies. Template letters and adrenal crisis action plans should be issued to schools informing them that the child has AI and what to do in an emergency. Having a dedicated clinical nurse specialist or key worker as a point of contact can be extremely valuable.

Support Organizations

Several patient advocacy and support groups are available for young people with AI and their families. These organizations can play an invaluable role in education and in bringing together young people and families with similar conditions. An overview of several organizations

Emergency kits

Your specialist treatment centre will issue you with emergency medication for your kit, one of which the child should carry at all times. Another should be kept at the child's nursery, school or college.



Each emergency kit should contain:

- 1× vial of hydrocortisone as Efcortesol®
- 1× 2 ml syringe
- 2× blue needles
- 1× tube of Glucogel® gel with instructions for use
- 1× leaflet '*How to give an emergency injection of Efcortesol®*'
- 1× steroid card filled in with child's details which you should keep in a specially labelled container.

You should check the expiry dates of Efcortesol® and Glucogel® and order replacements from your GP before they pass their expiry date.

Figure 9.27 Description of an emergency pack that can be carried by children and young people with adrenal insufficiency and details of the 'MyCortisol' app. *Source:* Modified with permission from an original leaflet and app ('MyCortisol') produced by Great Ormond Street Hospital for Children NHS Foundation Trust (2015).

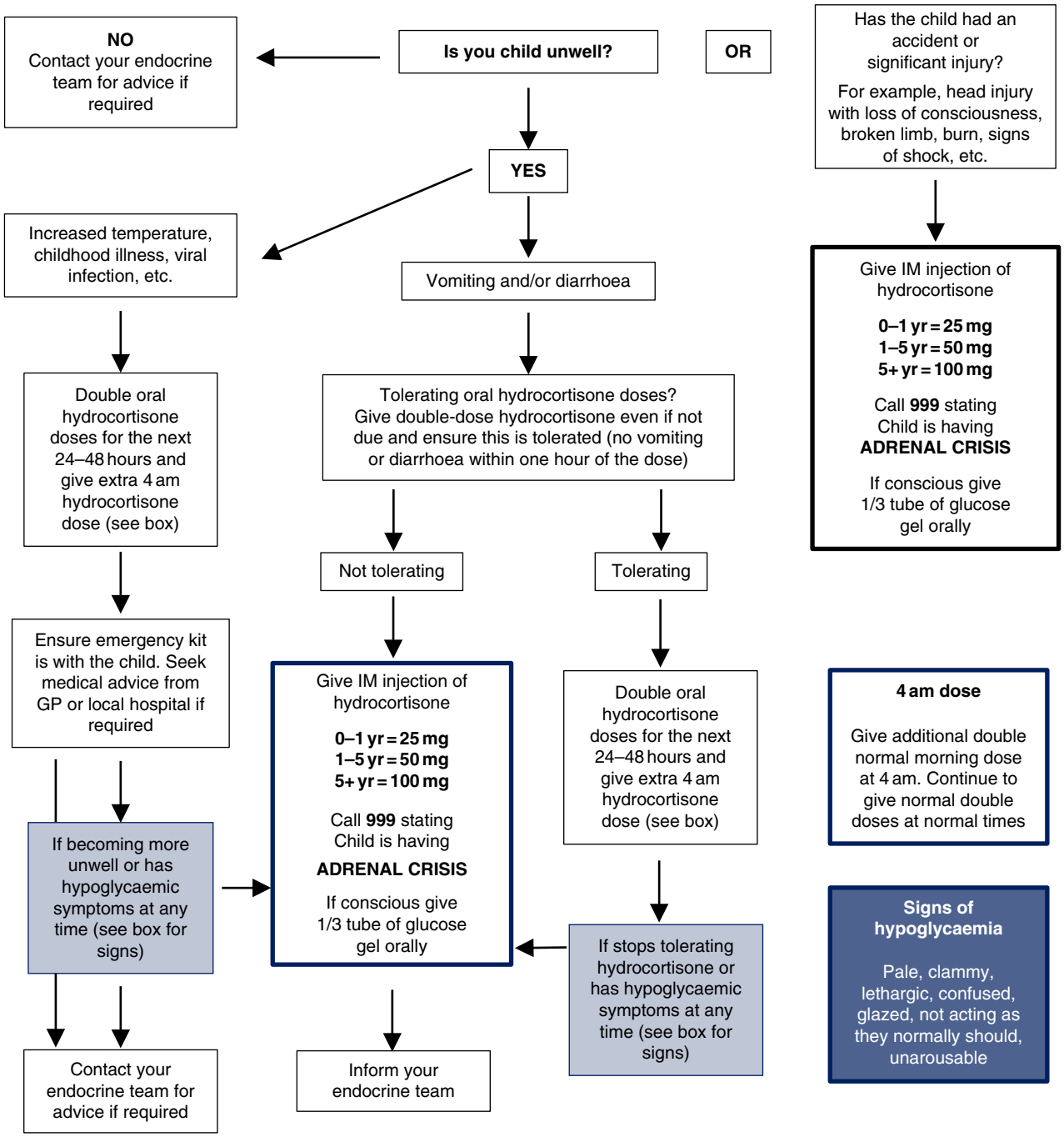


Figure 9.28 Overview of an illness management flow sheet for patients with adrenal insufficiency. *Source:* Modified with permission from an original leaflet ('Parents – Replacement Hydrocortisone Flow Sheet') produced by Great Ormond Street Hospital for Children NHS Foundation Trust (2015).

is provided in Table 9.9, although many other excellent local and national groups exist. Several useful medical resources are also listed.

Transition and Long-Term Care

As children and young people with adrenal disorders grow up, careful transition to adult services is required. Many pediatric adrenal diagnoses are rare and most adult endocrinologists will not be familiar with them. Adult services may not be structured with the same support, especially if people move to different places or are not engaged with specialist centres. Dedicated clinical nursing support may not be as accessible as in childhood.

Coupled with this, many young people find the need for lifelong treatment challenging. Glucocorticoid

Table 9.9 Selected websites for support groups and medical resources.

Key support groups	
Addison's Disease Self-Help Group (ADSHG)	http://www.addisons.org.uk/
AdrenalNET	http://adrenals.eu/
ALD Life	http://www.aldlife.org/
CAH Support Group Australia	http://www.cah.org.au/
CAH Education and Support Network	http://www.congenitaladrenalhyperplasia.org/
CARES Foundation (USA)	http://www.caresfoundation.org/
Living with CAH (UK)	http://www.livingwithcah.com/
National Adrenal Diseases Foundation	http://www.nadf.us/
Useful medical resources	
American Academy of Pediatrics (AAP)	https://www.aap.org/
Australasian Paediatric Endocrine Group (APEG)	https://apeg.org.au/
British Society of Paediatric Endocrinology and Diabetes (BSPED)	https://www.bsped.org.uk/
GeneReviews	http://www.genereviews.org/
European Society of Endocrinology (ESE)	http://www.ese-hormones.org/
Pediatric Endocrine Society (PES)	https://www.pedsendo.org/
Society for Endocrinology	http://www.endocrinology.org/

Resources are listed alphabetically.

replacement may not fit with their lifestyle and they resent having a chronic condition. Treatment may be blamed for weight gain or side effects but non-compliance is associated with considerable risk of an acute adrenal crisis so ongoing engagement, education and emotional/psychological support are important.

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10

The Parathyroid and Disorders of Calcium and Bone Metabolism

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KEY LEARNING POINTS

- Vitamin D deficiency should be excluded before other causes of metabolic bone disease can be made.
- Rickets is a disorder of the growing skeleton and can be due not only to nutritional deficiency of vitamin D but any disease that causes a lack of phosphate at the growth plate.
- Experienced pediatricians should manage hypophosphataemic rickets in childhood.
- The diagnosis of childhood osteoporosis should follow the latest International Society for Clinical Densitometry Official Positions.
- Early treatment of perinatal or infantile hypophosphatasia can dramatically improve outcome.

Introduction

Calcium and phosphate have an important role in maintaining neuromuscular and cell function and also have a structural role as components of bone. Their physiology is complex and the mechanisms that control them are many and can be disrupted in many ways.

Many of the causes of mineral and bone disorders have a genetic basis and much of the discussion in this chapter will relate to these. These conditions may be rare but they have particular value in shedding light on physiology. They will not all be discussed in detail but they and the genes that determine them are referred to by their Online Mendelian Inheritance in Man (OMIM) numbers so that further details with the relevant references can be obtained directly from the website http://www.ncbi.nlm.nih.gov/omim. Abbreviations of genes are shown in italics and proteins in plain text. More detailed descriptions and case histories can be obtained from Allgrove and Shaw [1].

Physiology of Calcium and Bone Metabolism

Cations and Anions

Calcium

About 1200 g of calcium is present in a fully grown adult, 99% in bone. The remainder circulates in plasma in three fractions:

1) An ionized fraction that constitutes about 50% of the total and is maintained at a concentration of between 1.1 and 1.3 mmol/L (4.4–5.2 mg/dL). This determines optimal neuromuscular function and is maintained by the endocrine factors responsible for its stability.
2) Most of the remaining 40–50% circulates bound to albumin and hypoalbuminaemia may reduce the total circulating concentration without affecting the ionized calcium.
3) The remainder circulates as complexes with other molecules such as citrate and sulphate.

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Companion website: www.wiley.com/go/dattani/brookcpe7

The control of ionized calcium in plasma is mainly under the influence of parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), both of which increase concentrations. Calcitonin (CT) and parathyroid hormone-related peptide (PTHrP) have a lesser influence. Fibroblast growth factor 23 (FGF23) also has a significant effect on phosphate metabolism and should be regarded as a classic hormone.

Ionized calcium can be measured directly by most blood gas analysers but laboratories usually measure total calcium and albumin and make an adjustment to allow for the albumin concentration. Such adjustments usually have little clinical relevance, unless severe hypoalbuminaemia is present. A suitable estimate can be obtained from the formula:

$$\text{CaCorr} = \text{CaTotal} + \left(41 - \left[\text{Alb}\right]\right) * 0.017),$$

where CaTotal is the observed total calcium (in mmol/L) and [Alb] is the plasma albumin in g/L (for calcium measured in mg/dL, the albumin correction factor is 0.068).

Control of plasma calcium results from a combination of intestinal absorption, renal tubular reabsorption and, where necessary, equilibration with bone stores. The mechanisms by which calcium and magnesium are transported across both intestinal and renal epithelial cells include para- and trans-cellular processes that have been reviewed by Hoenderop et al. [2] and by Dias de Barboza et al. [3]. The paracellular mechanisms occur via tight junctions made up of a combination of proteins including, among others, the claudins. The most important of these for calcium transport are claudins 2 (CLDN2) (*300520), 12 (CLDN12) (*611232) and 15 (CLDN15) (*615778) that facilitate the passive transport of calcium across intestinal cells, although CLDN2 and CLDN12 are also influenced by $1,25(OH)_2D$ [4]. Absorption is determined by the concentration and electrochemical gradients across the tight junctions, mainly in the upper small bowel.

Paracellular renal tubular reabsorption of calcium and magnesium occurs principally in the thick ascending loop of Henle (TALH) where three relevant claudins are present. Claudin 16 (CLDN16) (*603959) (formerly known as paracellin 1) and claudin 19 (CLDN19) (*610036) facilitate the passive transport of both calcium and magnesium across intestinal and renal tubular cells. Claudin 14 (CLDN14) (*605608) inhibits CLDN16 and CLDN19 cation channels and is itself upregulated by the calcium-sensing receptor (CaSR) [5]. Thus hypocalcaemia stimulates calcium reabsorption and hypercalcaemia the reverse.

Active trans-cellular transport occurs via separate processes at the luminal surface, in the cytosol and at baso-lateral extrusion [2]; all are influenced by different proteins. At the luminal surface, two members of the transient receptor potential (TRP) channel protein family, TRPV5 (*606679) and TRPV6 (*606680), have a major role in controlling calcium influx acting as 'gatekeepers' to facilitate the ingress of calcium into the cells. They demonstrate a negative feedback mechanism whereby once the intracellular calcium concentration rises beyond a certain point, further absorption is inhibited.

TRPV6 is the most important in the intestine and is stimulated by $1,25(OH)_2D$ but a second protein, $Ca_v1.3$, also promotes calcium absorption [3]. TRPV6 is found mainly in the upper part of the intestine, particularly duodenum, and $Ca_v1.3$ is present in higher concentrations in the jejunum. It is thought that TRPV6 operates mainly in the polarizing conditions of the fasting state in the upper small bowel while $Ca_v1.3$ is more responsible for calcium absorption in the fed, depolarized state in the lower small bowel, and that they work in tandem depending on the time from the last meal [3].

Cytosolic diffusion is largely facilitated by two intracellular proteins, calbindin_{28K} (CALB1) (*114050) and calbindin_{9K} (CALB3) (*302020), that bind calcium and transport it across the cell cytoplasm.

Extrusion of calcium at the baso-lateral surface is an active process facilitated by both an ATP-dependent Ca^+-ATPase (PMCA1b) (*108731) and a Na^+/Ca^+ exchanger (NCX1) (*182305). In the small intestine the former is more important and is under the influence of $1,25(OH)_2D$. It has a novel partner protein, 4.1R, with which it co-locates, but its function is not understood [3]. Absorption of calcium may be reduced in the presence of large quantities of calcium-binding agents such as phytate or oxalate.

Excretion is mainly via the kidney and is influenced by a number of dietary factors that include sodium, protein and acid loads, all of which increase calcium excretion [6]. Most reabsorption occurs largely passively in conjunction with sodium in the proximal tubule (70%) but a further 20% is absorbed in the TALH by the same paracellular mechanism as in the intestine [5]. Passive reabsorption occurs only where CLDN16 is present. 5–10% is reabsorbed in the distal tubule, under hormonal control [7] involving trans-cellular mechanisms similar to those in the intestine. TRPV5 is thought to be the more important protein at the luminal surface. Cytosolic diffusion is also facilitated by calbindins. NCX1 is the more important protein at the baso-lateral surface and is under the influence of PTH (Figure 10.1).

During childhood, particularly during the phases of rapid growth in infancy and adolescence, the highest proportion of ingested calcium is absorbed to allow bone mineralization.

Urinary calcium excretion is most easily measured by assessing the ratio of calcium to creatinine (Ca/Cr) in a mid-morning urine specimen. This should not normally exceed 0.7 mmol/mmol (0.25 mg/mg) [8]. Excess calcium excretion occurs in hypercalcaemic conditions associated with hyperparathyroidism and vitamin D excess, as well as in those that are not associated with hypercalcaemia, such

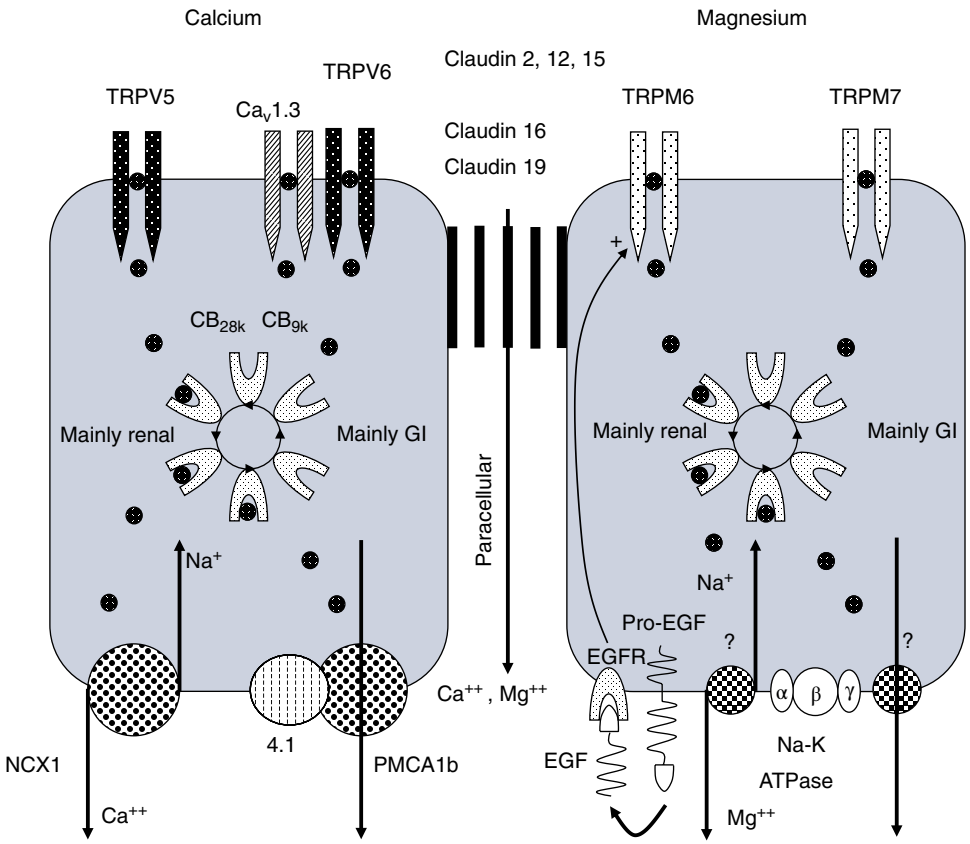


Figure 10.1 Schematic representation of divalent cation transport across gastrointestinal and renal cells. Calcium is shown on the left and magnesium on the right. The luminal surface is at the top and the baso-lateral surface at the bottom.

as activating mutations of the CaSR and distal renal tubular acidosis, all of which may cause nephrocalcinosis. Primary renal tubular disorders are also frequently associated with hypercalciuria and nephrocalcinosis or nephrolithiasis. Hypercalcaemia associated with inactivating mutations of the CaSR or hypocalcaemia caused by hypoparathyroidism results in low urinary calcium excretion.

Magnesium

This cation, which circulates in plasma at a concentration of 0.7–1.2 mmol/L (1.7–2.9 mg/dL), is important because it is required for normal PTH secretion as a component of the guanine nucleotide-binding protein alpha-11 (GNA11), second messenger of the CaSR (see below) [9]. It is absorbed mainly in the small intestine by passive absorption but active absorption occurs in the apical enterocytes of the colon by trans-cellular mechanisms very similar to those of calcium but mediated by TRPM6 (*607009) and TRPM7 (*605692) [10]. The mechanisms that control these processes are less well understood than those of calcium but active transport across luminal cells is similar. One form of autosomal recessive **hypomagnesaemia with secondary hypocalciuria** (HOMG1) (#602014) is caused by mutations in *TRPM6* [11]. Passive absorption is not affected in this condition and calcium excretion is low.

Renal tubular transport occurs by both para- and trans-cellular mechanisms: following filtration by the renal glomerulus, passive reabsorption occurs along the TALH at the same sites as those of calcium. Mutations in the *CLDN16 (paracellin 1)* gene (*603959) result in impaired magnesium and calcium reabsorption and are the cause of the **hypomagnesaemia, hypercalciuria and nephrocalcinosis** (HOMG3) syndrome (#248250) [12]. Further down the renal tubule, a similar tight junction protein, CLDN19 (*610036), permits transport of calcium and magnesium in the collecting ducts [12]. Mutations in this gene cause **renal hypomagnesaemia with ocular involvement** (HOMG5) (#248190).

Active trans-cellular absorption occurs mainly in the distal convoluted tubule (DCT) [13]. TRPM6 is the most specific of the proteins responsible for transport across the luminal surface and renal reabsorption is also affected in HOMG1. A similar protein, TRPM7, is involved in magnesium transport but is more universally distributed. The activity of TRPM6 is influenced by epidermal growth factor (EGF) (*131530), a soluble protein derived from its precursor, pro-EGF, which is membrane bound on the baso-lateral membrane of the renal tubules. Once EGF has been cleaved from

pro-EGF, it interacts with its receptor (EGFR (*131550)), which, among its other actions, stimulates magnesium absorption via TRPM6 on the luminal surface. Mutations in *EGF* disrupt the baso-lateral sorting of Pro-EGF and cause understimulation of TRPM6 and impaired magnesium reabsorption [14] resulting in **isolated recessive renal hypomagnesaemia** (IRH) or HOMG4 (#611718).

Cytoplasmic transfer is probably effected by intracellular proteins such as CALB1, but the mechanisms are not understood. At the baso-lateral membrane, active transport occurs by means of the Na^+,K^+,-ATPase pump. The mechanism by which this takes place is not fully understood but mutations in *FXYD2* (*601814), which codes for the γ-subunit of this protein, cause autosomal dominant **renal hypomagnesaemia associated with hypocalciuria** (HOMG2) (#154020) [12] and sometimes cause severe symptomatic hypomagnesaemia. CNNM2 (*607803) is a member of the cyclin M family of proteins also located on the baso-lateral membrane and mutations in this gene cause **hypomagnesaemia with seizures and mental retardation** (HOMG6) (#613882). The thiazide-sensitive sodium chloride co-transporter (NCC) is also involved in magnesium transport and mutations in *SLC12A3* (*600968) cause **Gitelman's syndrome** (#263800) in which hypermagnesuria is a feature [12]. Raised urinary magnesium excretion is also present in some cases of **Bartter's syndrome**, which is caused by a variety of mutations affecting chloride and sodium reabsorption in the loop of Henle, which has a specific role in magnesium transport in the kidney [13]. Renal tubular transport of magnesium can be increased by several non-genetic causes including diuretics, diabetic ketoacidosis, gentamicin, mercury-containing laxatives, transplanted kidney, urinary tract obstruction, the diuretic phase of acute renal failure and cisplatin.

Phosphate

A fully grown adult contains ~700 g phosphate of which about 80% is contained in the bone. 45% (9% of the total) is present in skeletal muscle, 54.5% in the viscera and 0.5% in extracellular fluid. Most phosphate is present in inorganic form but it plays a crucial part in many intracellular processes. Phosphate circulates in plasma as phospholipids, phosphate esters and free inorganic phosphate (Pi). Plasma Pi concentrations are not controlled as tightly as those of calcium and reflect the fluxes of phosphate entering and leaving the extracellular pool. In contrast to calcium, phosphate concentrations vary considerably during life, being highest during phases of rapid growth. Phosphate concentrations in premature infants are normally above 2.0 mmol/L (6.4 mg/dL), fall to 1.3–2.0 mmol/L (4.2–6.4 mg/dL) during infancy and childhood and to 0.7–1.3 mmol/L (2.2–4.3 mg/dL) in young adults.

Phosphate transport across membranes is controlled by a series of sodium-dependent active transport mechanisms (Na/Pi co-transporters). Three types are known to exist [15]:

1) Type 1 is present in renal tubular brush borders but is not thought to have a major role in renal tubular reabsorption of phosphate (TRP).
2) Type 2, which has three subtypes, 2a, 2b and 2c, is the most important in regulating phosphate absorption and reabsorption.
3) Type 3 is present in many tissues but is thought to have more of a 'gatekeeping' role.

Phosphate is readily absorbed throughout the small bowel by passive and active mechanisms, 70% by type 2b Na/Pi co-transporter and the remainder by passive absorption. This active transport is stimulated directly by $1,25(OH)_2D$ and therefore indirectly by hypocalcaemia and PTH. Since hypophosphataemia is a powerful stimulant of 25-hydroxyvitamin D-1-alphahydroxylase (1α-hydroxylase), phosphate deficiency itself stimulates increased absorption. The total amount absorbed is dependent on the dietary phosphate load and may be inhibited by phosphate-binding agents such as calcium acetate (Phosex®) or carbonate (Tetralac®) or sevelamer (Renagel®). These are of value in hyperphosphataemic states such as chronic renal failure when phosphate absorption needs to be limited.

Regulation of plasma phosphate occurs in the renal tubule via the type 2a and 2c Na/Pi co-transporters of which 2c (*609826) is the most important. Its activity is dependent mainly on the activity of FGF23, a major phosphotonin that stimulates phosphate excretion (see below for a more detailed discussion of FGF23). Excess FGF23 results in hypophosphataemia and low concentrations are associated with hyperphosphataemia. This has significant clinical implications in a number of situations. 85–98% of the filtered load of phosphate is reabsorbed, mostly in the proximal renal tubule, which represents ten times the amount absorbed in the intestine. The TRP by the renal tubule is a saturable process determined both by the filtered load, which is itself determined by glomerular filtration rate (GFR), by plasma concentration and by hormonal factors, particularly PTH and FGF23, both of which increase phosphate excretion.

Assessment of phosphate excretion is crucial to diagnosing some conditions, particularly hypophosphataemic rickets. It is most easily assessed by measuring the fractional excretion of phosphate ($FEPO_4$), which is best described as the ratio of the clearance of phosphate to the clearance of creatinine and requires measurement of plasma and urinary phosphate and creatinine on single samples taken simultaneously. It makes the assumption

that creatinine clearance approximates to GFR but does not require timed urine samples. $FEPO_4$ is calculated according to the formula:

$$FEPO_4 = [UPO_4]/[PPO_4] \times [PCreat]/[UCreat]$$

where all the results for phosphate and creatinine are expressed in the same units.

TRP is 1-$FEPO_4$ and is usually expressed as a percentage. Values are normally above 85% and frequently approach 98% in children. In hyperphosphaturic conditions, the value may be below 50% but this is dependent on the filtered load of phosphate: the lower the plasma concentration, the greater the proportion that can be reabsorbed. A more precise measure of renal tubular phosphate handling, which eliminates any effect of plasma phosphate, can be obtained by calculating the theoretical tubular maximal phosphate threshold as a function of GFR ($TmPO_4$/GFR). This is most easily obtained by the nomogram of Walton and Bijvoet [16] from the plasma phosphate concentration and the $FEPO_4$. $TmPO_4$/GFR is reduced in hyperparathyroidism and phosphate-losing conditions and increased in hypoparathyroidism. It is higher in children and adolescents than in adults [17].

Hormones and Other Calciotropic Agents

Alkaline Phosphatase

This enzyme is present in several tissues and exists in three isoforms, intestinal (IALP) (*171740), placental (PLALP) (*171810) and tissue non-specific (TNSALP) (*171760). A gene on chromosome 2q34-37 codes for the first two and a gene on chromosome 1p36.1-p34 codes for the last [18]. Different post-translational modifications of TNSALP result in three tissue-specific forms found in the bone, liver and kidney that can be distinguished by their different isoelectric points and heat lability, the bone-specific form (bTNSALP) being the least stable.

bTNSALP is present in osteoblasts and promotes bone mineralization. Circulating TNSALP is largely derived from liver and bone. Concentrations in plasma during childhood reflect growth rate [19] and are raised in the presence of rickets, **Juvenile Paget's Disease** (#239000) and transient hyperphosphatasia of infancy [20]. Alkaline phosphatase has to be anchored to cell surfaces by glucosylphosphoinositol (GPI) and, in the presence of mutations that result in abnormalities of GPI, ALP is raised because soluble ALP leaks out into the blood stream. Therefore ALP is also raised in hyperphosphatasia with mental retardation, of which six different types are described (#239300; #614749; #614207; #615716; #616725; #616809) due to different mutations in GPI synthesis [21]. Low concentrations are seen in hypophosphatasia, which results from mutations in TNSALP. A database that keeps track of these mutations (currently 307) has been established and can be accessed at http://www.sesep.uvsq.fr/Database.html.

The principal function of TNSALP in bone is to dephosphorylate pyrophosphate (PPi) to Pi. It has several other clinically relevant substrates, including vitamin B6 (pyridoxal-5 phosphate, PLP), which has to be dephosphorylated before it can traverse the blood–brain barrier before being rephosphorylated in brain tissue. One consequence of this is that pyridoxine-dependent seizures may be manifest in severe forms of hypophosphatasia. Another enzyme, ecto-nucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1), does the reverse of TNSALP. Since PPi is an inhibitor of bone mineralization, this, together with the lack of TNSALP to provide Pi for hydroxyapatite formation, leads to variable degrees of poor mineralization in hypophosphatasia. Mutations in *ENPP1* result in **generalized arterial calcification of infancy** (GACI) (#208000) [22], which has a mortality rate of 85% in infancy. Survivors often develop a form of **autosomal recessive hypophosphataemic rickets type 2** (ARHR2) (#613312) [22].

Parathyroid Hormone (PTH)

PTH (*168450) is a single-chain, 84-amino acid, polypeptide hormone encoded by *PTH* on chromosome 11. It is synthesized by the parathyroid (PT) glands from prepro-PTH, which has an additional 31 amino acids, in the ribosomes, where the initial 25-amino acid 'pre' sequence acts as a signal peptide to aid transport through the rough endoplasmic reticulum. The 'pre' sequence is cleaved, and pro-PTH then travels to the Golgi apparatus where the 6-amino acid 'pro' sequence is cleaved to yield the mature hormone, which is stored in secretory vesicles that fuse with the plasma membrane prior to secretion of the hormone [23]. Very little PTH is stored within the glands and most of the secreted hormone is newly synthesized. Mutations in *PTH* have been described as a cause of isolated hypoparathyroidism [24].

Only the first 34 N-terminal amino acids are required for full activity and the function of the remainder of the molecule is not understood. The half-life of PTH in the circulation is 1–2 minutes [23]. The molecule is cleaved at various sites, which results in a number of fragments that can be identified in the circulation. The best assays measure 'intact' PTH; they measure physiological concentrations of PTH, correlate well with bioactivity and ignore the inactive fragments. Normal concentrations of PTH in the circulation are about 1–6 pmol/L (10–60 pg/mL) but vary depending on the assay.

Control of PTH Secretion

The CaSR Receptor Complex PTH is secreted in response to changes in ionized calcium. A CaSR (+601199) is present in many tissues, particularly the PT glands and renal tubule, as well as bone, cartilage, and other tissues [25]. The *CaSR* gene is located on chromosome 3q13.21 and the CaSR is a large molecule consisting of 1078 amino acid residues. Approximately 610 of these form the extracellular calcium-binding domain, 250 comprise the seven transmembrane domain and another 210 comprise the intracellular cytosolic component. Ca++ binds to the extracellular domain in a complex manner and influences PTH secretion by both phospholipase Cb and G-protein second messengers. As a consequence, PTH secretion changes in a sigmoidal fashion in response to acute changes in plasma calcium (Figure 10.2), and there is a continuous tonic secretion of PTH, which maintains plasma-ionized calcium at whatever level is 'set' by the CaSR [26]. Magnesium also binds to the CaSR and influences PTH secretion in a fashion similar to that of calcium. Severe magnesium deficiency inhibits PTH secretion because it is a component of the GNA11 second messenger (see below).

Mutations within *CaSR* result in either inactivation or activation of the receptor, which result in hyper- and hypocalcaemia, respectively. Inactivating mutations cause insensitivity to calcium, which shifts the curve of PTH secretion in response to plasma calcium to the right (Figure 10.2). As a consequence, PTH secretion is switched off at a higher concentration than normal, and hypercalcaemia results [25]. The receptors are also present in the renal tubule, and renal calcium excretion is therefore reduced. Activating mutations of *CaSR* give rise to a form of **hereditary autosomal dominant hypoparathyroidism** (ADH, HYPOC1) (#601198) [27], while inactivating mutations cause either **familial benign hypercalcaemia** (FHH1) (#145980) or **neonatal severe hyperparathyroidism** (NSHPT) (#239200) [28].

Many of the mutations found in FBH are clustered around the aspartate- and glutamate-rich regions of the extracellular domain of the molecule and it has been postulated that this region contains low-affinity binding sites for calcium. Many of the FBH kindreds have unique mutations. Mutations have also been detected within the transmembrane domain but only rarely within the intracellular domain. Most activating mutations that cause ADH are also present within the extracellular calcium-binding domain. Several hundred mutations, some of which are polymorphisms, have been described and an online database has been established to keep track of them (http://www.casrdb.mcgill.ca).

The CaSR complex has two other components, the GNA11 (*139313) and the adaptor-related protein complex 2, sigma-1 subunit (AP2S1) (*602242), the genes for which are located on chromosomes 19p and 19q13,

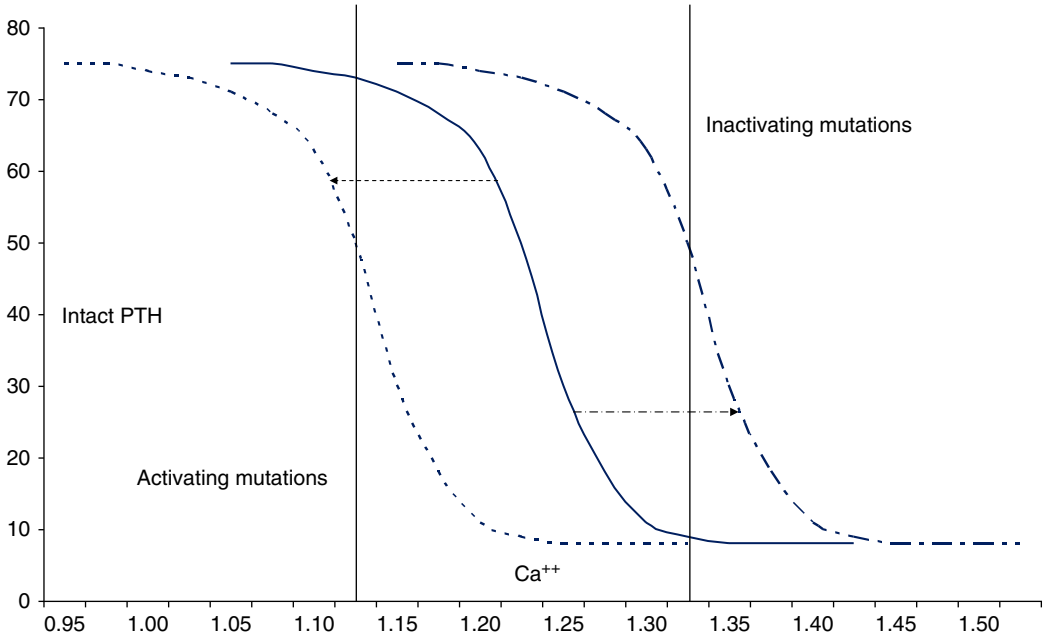


Figure 10.2 Schematic representation of the sigmoidal relationship between ionized Ca (Ca^{++}) and intact PTH secretion. The vertical lines represent the normal range of Ca^{++}. Also shown is the effect of inactivating mutations (right shift) and activating (left shift) of the *CaSR*. The degree to which these shifts occur is dependent on the mutation involved. Adapted from the original data of Conlin et al. [26]. Reproduced with permission of Oxford University Press.

respectively. Mutations within these loci result in clinical syndromes very similar to those resulting from inactivating mutations of the CaSR itself, although no clinical syndromes are associated with activating mutations of *AP2S1* [29]. The three variants of FBH linked to chromosome 3q, 19p and 19q have therefore been referred to as FHH types 1–3, the last of which is known as the Oklahoma variant.

The Parathyroid (PT) Glands The PT glands, usually four in number, are derived from the third (lower glands) and fourth (upper glands) branchial arches [30]. Transcription factors involved in their development [30, 31] include some, such as *Hoxa3* (thyroid and thymus) (*142954) and *GATA3* (auditory nerves, kidney) (*131320), which are involved in the development of other structures. Several genes, including *Tbx1* (thymus, cardiac outflow tract, and the face) (*602054) and *UDF1L*, are located on the long arm of chromosome 22. Mutations within them result in congenital hypoparathyroidism, which may be either isolated or associated with other conditions such as the **hypoparathyroidism, deafness and renal anomalies** (HDR) (#146255) **syndrome** and the **22q deletion syndrome** (previously known as the CATCH 22 complex), of which the **DiGeorge syndrome** (DGS) (#188400) is part. Mutations in *tubulin-specific chaperone E (TBCE)* (*604934) encompass a group of disorders including **Sanjad-Sakati** (#241410) and **Kenny-Caffey** (#244460, #127000) syndromes that include not only hypoparathyroidism but also short stature, ocular abnormalities and mental retardation.

Mitochondrial genes are also involved in PT gland development and mutations, which show maternal inheritance, give rise to various syndromes such as **Kearns-Sayre** (#530000) and **MELAS** (#540000).

Finally, mutations in *autoimmune regulator (AIRE1)* (*607358), the first gene described to cause inherited autoimmune disease, results in **autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy** (APECED, APS1) (#240300) syndrome in which hypoparathyroidism is usually the first endocrine manifestation.

The homologue of drosophila *glial cells missing 2 (GCM2)* (*603716) is a highly conserved gene necessary for PT gland development but it has no other known function. Mutations cause autosomal dominant [32] or recessive [33] **familial isolated hypoparathyroidism** (FIH) (#146200). The *SRY-related HMG-box gene 3 (SOX3)* (*313430) located on the X-chromosome is thought to be involved in PT gland development and mutations in it may be responsible for **X-linked recessive FIH** (%307700). Destruction of the glands may occur as a result of thyroid or PT surgery, autoantibodies or infiltration (e.g. with iron in β-thalassaemia).

Actions of PTH

PTH acts on the bone and kidney; in the bone it promotes mineralization by an action on osteoblasts when present at physiological concentrations. During phases of hypocalcaemia, when PTH concentrations increase, its main effect is on the osteoclast via osteoblasts, where it stimulates bone resorption within the basic modelling unit (BMU) via the RANKL/RANK system (see below). The two processes do not occur independently and increases in bone resorption are accompanied by stimulation of osteoblast activity via a series of paracrine and autocrine mechanisms that increase bone turnover.

In the nephron, most of the filtered calcium is reabsorbed passively in the proximal tubule via a paracellular mechanism (see above). In the convoluted and straight parts of the proximal renal tubule, PTH stimulates 25-hydroxyvitamin D 1α-hydroxylase (1α-hydroxylase) to convert 25OHD to its active metabolite, $1\alpha,25(OH)_2D$. PTH promotes calcium and magnesium trans-cellular reabsorption in the distal nephron. Phosphate excretion increases in response to PTH to allow excess phosphate that has been removed from bone with calcium following PTH-stimulated bone resorption to be excreted. PTH also stimulates renal excretion of bicarbonate and amino acids. Thus hyperparathyroidism results in a mild acquired form of **Fanconi** syndrome.

Mechanisms of Action of PTH

The PTH Receptor PTH acts through two receptors, the first and principal one is PTH type r Receptor (PTH1R) (also called PTH/PTHrP) receptor (*168468), which has equal affinity for PTH and PTH-related peptide (PTHrP). It consists of 593 amino acids encoded by a gene on the long arm of chromosome 3 [34]. It has an extracellular binding domain of 190 residues, a seven transmembrane domain and a cytosolic component of 134 residues. Inactivating and activating mutations of the *PTH1R* have been described, which result in **Blomstrand lethal chondrodysplasia** (#215045) and **Jansen disease** (#156400), respectively. A second PTH2 receptor (PTH2R) is present in the central nervous system but PTHrP is not a ligand for it.

Intracellular Signalling Intracellular signalling occurs by coupling the cytosolic component of the PTHR1 to G-protein second messengers, Gs and Gq [35, 36]. These are heterotrimeric, consisting of α, β and γ subunits. In the resting state, they are associated, and the Gsα subunit is bound to GDP (Figure 10.3a). Binding of the ligand to the receptor results in GDP being exchanged for GTP and dissociation of the Gsα subunit

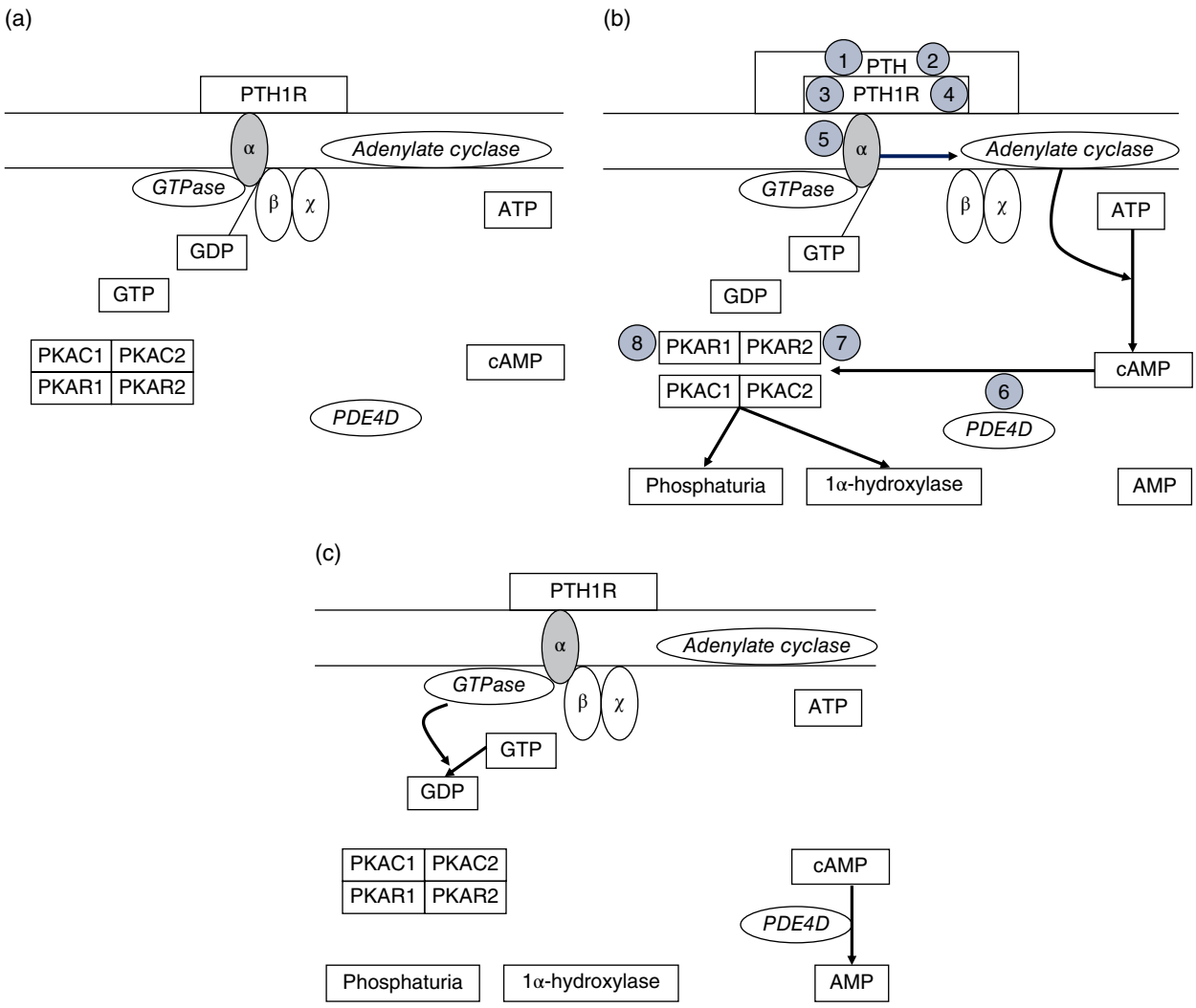


Figure 10.3 Simplified representation of mechanism of action of PTH in relation to the G-protein second messenger. In the resting state (a), the Gsα subunit is bound to GDP and associated with the β,γ subunit. When PTH binds to the receptor (b), GDP is exchanged for GTP, which causes dissociation of the Gsα subunit from the β,γ subunit. The Gsα subunit is then free to stimulate membrane bound adenylate cyclase, which increases intracellular cAMP and which then has its effects via protein kinases. The intrinsic GTPase activity associated with the Gsα subunit then hydrolyses GTP back to GDP (c), which causes reaggregation of the G-protein. At the same time, PDE4D phosphodiesterase inactivates the cAMP to AMP and the situation reverts to the quiescent state (a). In this diagram the less important Gq second messenger, which acts via inositol triphosphate (IP_3), is not shown. Also shown in shaded circles are the conditions that occur as a result of mutations within relevant genes:

1) PTH gene inactivation (primary hypoparathyroidism).
2) PTH gene activation (parathyroid tumours).
3) PTHR1 receptor activation (Jansen disease).
4) PTHR1 inactivation (Blomstrand chondrodysplasia).
5) GNAS1 inactivation (PsHP1a).
6) PDE4D activation (ACRDYS2).
7) PRKAR1A inactivation (ACRDYS1).
8) PRKAR1A activation (Carney complex).

from the β,γ complex. The Gsα is then free to stimulate adenylate cyclase, which results in an increase in intracellular cAMP that activates the actions of PTH through specific protein kinases (Figure 10.3b) such as PRKAR1A (*188830). The latter consists of regulatory and catalytic subunits (PKAR and PKAC) that, in the resting state, are associated and inactive. The regulatory and catalytic components are dissociated by cAMP and become active. Inactivation of cAMP is effected by phosphodiesterase type 4d (PDE4D). Activating mutations of *PDE4D* prevent this dissociation and cause **acrodysostosis type 2** (ACRDYS2) (#614613) while inactivating mutations of *PRKAR1A* cause **ACRDYS1**

(#101800) [37]. Resistance to hormone action, particularly PTH, may be a feature of either of these conditions, especially ACRDYS1. Inactivating mutations of *PDE4* and activating mutations of *PKAR1A* cause adrenal hypertrophy and the **Carney complex**, respectively.

Intrinsic GTPase activity associated with the Gsα subunit hydrolyses GTP to GDP, which causes reassociation of the components of the G-protein. At the same time, phosphodiesterases, particularly PDE4D, inactivate cAMP to AMP and the cell reverts to its resting state (Figure 10.3c). This mechanism is common to several hormones, including thyroid-stimulating hormone (TSH), gonadotropins and growth hormone-releasing hormone (GHRH) [35].

The Gsα subunit is encoded by *GNAS1*, (*139320) located on chromosome 20q13.3. This complex gene contains 13 exons that encode the Gsα subunit itself plus another five exons that, by alternative promoter use and splicing, results in at least five different mRNA transcripts. In most tissues, these show biallelic expression but some of the transcripts are derived from either the maternal or paternal alleles (see under Pseudohypoparathyroidism). The Gqα subunit activates phospholipase Cb to generate inositol triphosphate (IP3), although this is a lesser effect than cAMP generation.

Several factors modify responsiveness to PTH. Mutations within *GNAS1* for the Gsα subunit of the G-protein second messenger cause resistance by preventing activation of adenylate cyclase. This results in some of the forms of **pseudohypoparathyroidism**. PTH can also modify responsiveness to itself. Acute infusions of PTH cause desensitization by uncoupling the receptor from the G-protein. Alternatively, in the presence of chronic hyperparathyroidism, downregulation of the receptors occurs as a result of reduction in the number of receptors.

Resistance to PTH has been assessed in earlier times by examining the effects of PTH on phosphate excretion (the Ellsworth-Howard test) or on cAMP production in either urine or plasma. It has not been possible recently to undertake such stimulation tests because PTH has not been available for use as a test agent. Since synthetic PTH (teriparatide) has been introduced for use in postmenopausal osteoporosis, it should theoretically be possible to use it for such purposes again, although the manufacturers have not made it available in small enough quantities to make it worthwhile using it for this purpose and as understanding of the genetic mechanisms underlying many of these conditions has advanced, the need for stimulation tests has declined.

Vitamin D

Vitamin D is a secosteroid that exists in two forms. Cholecalciferol is synthesized as a result of the action of ultraviolet (UV) light on 7-dehydrocholesterol. The UV light breaks the B ring of the steroid molecule to produce previtamin D, which is then converted to native vitamin D (cholecalciferol) by the action of body heat. Ergocalciferol is synthesized by plants and differs in having an extra double bond in the side chain but it is equipotent with cholecalciferol and metabolized similarly. The generic term vitamin D is used here to include both compounds.

Under normal circumstances, about 80% of vitamin D consists of cholecalciferol synthesized in skin, the remainder being acquired from dietary sources as both chole- and ergocalciferols. However, the amount of vitamin D synthesized in skin is dependent upon skin colour and exposure. Following synthesis, it becomes bound to a specific vitamin D-binding protein (DBP) and passes to adipose tissue and the liver for storage and further metabolism. Vitamin D does not have significant biological activity, which requires two hydroxylation steps, first at the 25 and then at the 1 position (Figure 10.4) [38].

All the steps in vitamin D metabolism are catalysed by cytochrome P450 enzymes (Figure 10.4), the first by vitamin D 25 hydroxylases. There are at least four different enzymes that have an influence on 25-hydroxylase activity. They are distinguishable by their different affinities and capacities and by their intracellular localization. The first, a low-affinity, high-capacity enzyme (CYP27A1) (*606530) is located in mitochondria but there are no reports of rickets resulting from mutations in this gene, although they do cause **cerebrotendinous xanthomatosis** (#213700). A second high-affinity, low-capacity enzyme (CYP2R1) (*608713), which may be of greater physiological significance, is located within hepatic microsomes. Rare cases are described of rickets associated with mutations in this gene (#600081) [39]. Two other enzymes, CYP3A4 (*124010) and CYP2J2 (*601258), probably also have some effect on 25-hydroxylase but are mainly involved in drug metabolism.

The resulting product, 25-hydroxyvitamin D (25OHD), circulates in plasma bound to the DBP and is the most abundant vitamin D metabolite, circulating in nanomolar concentrations. Assay of this compound gives a measure of vitamin D status. Its concentration varies depending on the supply of vitamin D and shows a considerable annual variation with a peak about six weeks after maximal exposure to sunlight. It has some weak activity, which is not normally of clinical significance, but may become so in the presence of vitamin D excess. Vitamin D 25 hydroxylase also catalyses the conversion of the synthetic vitamin D analogues, 1α-hydroxycholecalciferol (alfacalcidol) and 1α-hydroxyergocalciferol (doxercalciferol), to $1\alpha,25(OH)_2D$.

25OHD is metabolized to its active hormone $1\alpha,25(OH)_2D$ by 25-hydroxyvitamin D 1α-hydroxylase, which is active only against metabolites that are already

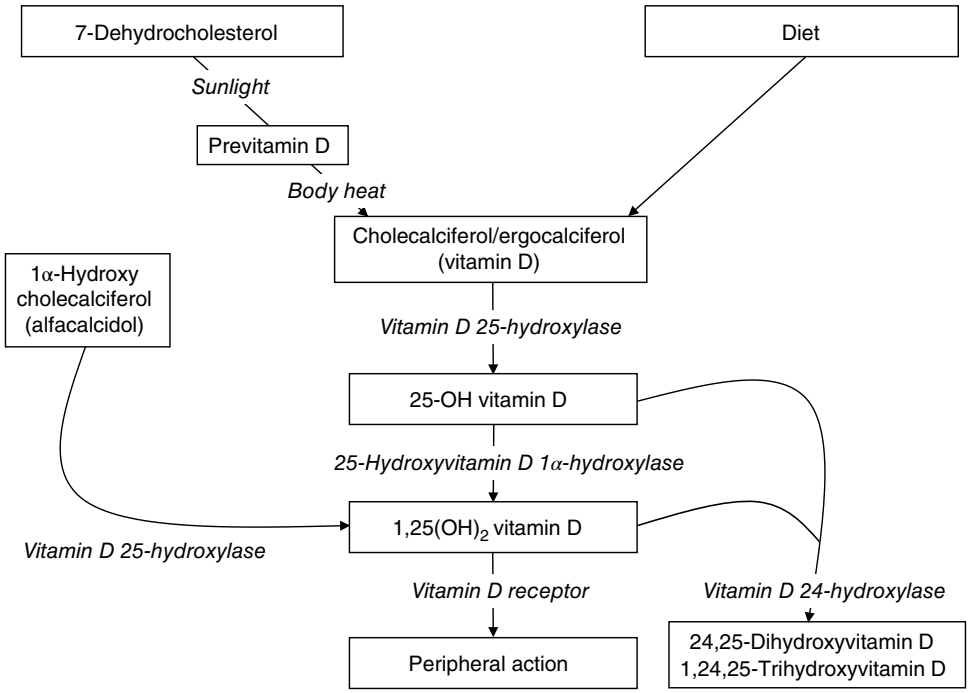


Figure 10.4 Diagrammatic representation of the principal steps involved in vitamin D metabolism.

hydroxylated at position 25 [40]. A single enzyme has been identified located in convoluted and straight portions of the proximal renal tubule. Activity is also present in osteoblasts, keratinocytes and lymphohaematopoietic cells, where 1α,25(OH)$_2$D may have an autocrine or paracrine role. During fetal life, 1α-hydroxylase activity is found in the placenta. In pathological states, it is present in the macrophages of sarcoid tissue and subcutaneous fat necrosis. It is a mitochondrial enzyme (CYP27B1) (*609506) consisting of 508 amino acids with considerable homology to other P450 enzymes. It is encoded by a single gene (*CYP27B1*) on chromosome 12q13.1-q13.3. Mutations in this gene are responsible for the condition known variously as **pseudovitamin D deficiency rickets** (PDDR), **vitamin D-dependent rickets type I** (VDDR-I), **Prader rickets** or **1α-hydroxylase deficiency** (#264700).

Activity of 1α-hydroxylase is stimulated by PTH via its cAMP/protein kinase actions. Hypocalcaemia stimulates 1α-hydroxylase activity but this effect is mediated by PTH and not directly. Plasma phosphate has a direct effect on 1α-hydroxylase activity, although there is some evidence to suggest that this may be modulated by growth hormone (GH); Calcitonin (CT) may also regulate the enzyme. Its activity is inhibited by FGF23 (see below) and high-dose corticosteroids.

1α,25(OH)$_2$D is a highly potent compound that circulates in picomolar concentrations. Its synthesis is tightly controlled by the plasma calcium concentration. In order to enable changes in 1α,25(OH)$_2$D to occur rapidly, a second enzyme, 25-hydroxyvitamin D 24-hydroxylase (25OHD 24-OHase) (*126065), exists. This is yet another cytochrome P450 enzyme that can use both 25OHD and 1α,25(OH)$_2$D as substrates to form 24,25-dihydroxyvitamin D (24,25(OH)$_2$D) and 1α,24,25-trihydroxyvitamin D (1α,24,25(OH)$_3$D), respectively. The role of this enzyme is probably to divert metabolism of 25OHD away from 1α,25(OH)$_2$D synthesis when this is not needed and to participate in the degradation of existing 1α,25(OH)$_2$D. It is inhibited by PTH and stimulated by 1α,25(OH)$_2$D and FGF23. Mutations in 24-hydroxylase have been reported to cause **infantile hypercalcaemia** (#143880) [41] and in adults, a tendency to renal stones [42].

1α,24,25(OH)$_3$D has limited potency (about 10% of 1α,25(OH)$_2$D) and is probably an intermediate degradation metabolite of 1α,25(OH)$_2$D. The role, if any, of 24,25(OH)$_2$D is uncertain but people of South Asian origin possess higher 25OHD 24-OHase activity than those of European origin [43] and this seems to contribute to their susceptibility to vitamin D deficiency rickets.

The Vitamin D Receptor (VDR)

1α,25(OH)$_2$D acts through a specific vitamin D receptor (VDR) [44] (*601769). It is a member of the steroid–thyroid–retinoid superfamily of nuclear receptors and, in many respects, is typical of this group with ligand binding, DNA binding, dimerization and transcriptional activation domains. It is encoded by a gene on chromosome 12 near the 1α-hydroxylase gene. The receptors are widely

distributed in the gut, PT glands, chondrocytes, osteoblasts, and osteoclast precursors. $1\alpha,25(OH)_2D$ plays a critical role in promoting calcium absorption in the small intestine, suppresses PTH secretion from the parathyroids, influences growth plate mineralization and stimulates differentiation of osteoclasts. In addition, there are receptors present in many tissues that are not directly related to calcium homeostasis such as the skin, breast, prostate and colon and it has been postulated that $1\alpha,25(OH)_2D$ may play a part in preventing cancers of these tissues.

Mutations in *VDR* occur throughout the molecule but particularly in either the ligand-binding (ligand-binding negative) or the DNA-binding (ligand-binding positive) domains. These mutations cause severe rickets and many individuals, especially those with defects in DNA binding, also have alopecia. Originally referred to as **vitamin D-dependent rickets type II** (VDRR-II), it is now more properly called **hereditary $1\alpha,25(OH)_2D$-resistant rickets** (HVDRR) (#277440). In another form of HVDRR, no mutations of the receptor have been identified but it is thought to be caused by overexpression of a nuclear ribonucleoprotein that binds with the hormone receptor complex to attenuate its action (%600785).

Fibroblast Growth Factor 23 (FGF23)

FGF23 (*605380) is mainly secreted by osteoblasts and osteocytes. It circulates in measurable amounts in plasma and is one of a number of fibroblast growth factors that function via fibroblast growth factor receptors (FGFRs) in a variety of tissues. It therefore acts as a classic hormone. The control and actions of FGF23 are summarized in Figure 10.5 [45]. FGF23 is the principal 'phosphotonin' that acts mainly via FGFR1c (*136350) to stimulate phosphate excretion via the type 2c Na/Pi cotransporter. It also inhibits 1α-hydroxylase, so that hyperphosphaturic conditions caused by raised FGF23 are not accompanied by the expected increase in $1,25(OH)_2D$, and stimulates 24-hydroxylase activity. *FGF23* is located on chromosome 12p13 and encodes a 251-amino acid peptide. It is inactivated by cleavage at a critical point between amino acids 179 and 180 into amino- and carboxy-terminal fragments. Activating mutations in *FGF23* cause **autosomal dominant hypophosphataemic rickets** (ADHR) (#193100) by preventing this processing, resulting in raised FGF23 in plasma; while inactivating mutations cause **hyperphosphataemic familial tumoral calcinosis type 2** (HFTC2) (#211900) since FGF23 can no longer inhibit phosphate reabsorption by renal tubules.

Protection against cleavage is conferred by O-glycosylation of the threonine at position 178. This is effected by UDP-polypeptide N-acetylgalactosaminyl transferase 3 (GALNT3) (*601756). Mutations in *GALNT3* also cause **familial tumoral calcinosis type 1** (#211900) because FGF23 is rendered inactive (see also FAM20C below).

Several other factors have a direct or indirect effect on FGF23. For the active receptor to be generated, another protein, Klotho (KL) (*611135), is required that combines with the receptor and allows it to be responsive to FGF23. *KL* is located on chromosome 13q12 and codes for a 1014-amino acid protein that contains two internal repeats. The mechanism by which it activates FGF23 is uncertain but it is thought to confer specificity of action to FGF23 within certain tissues, mainly kidney, PT and pituitary. Inactivating mutations of *KL* cause **hyperphosphataemic tumoral calcinosis type 3** (HFTC3) but with high circulating concentrations of FGF23 [46].

The *phosphate-regulating gene with homology to endopeptidases on the X-chromosome (PHEX*) (*300550) encodes a 749-amino acid and 7 transmembrane glycoprotein and is present in several tissues but not kidney [47]. It is responsible, via its endopeptidase activity, for the processing and cleavage of FGF23 to prevent hyperphosphaturia. The gene is located on the X-chromosome, and mutations cause classical **X-linked dominant hypophosphataemic rickets** (XLH) (#307800). Several hundred mutations have been described and an online database has been established (http://phexdb.mcgill.ca). It is not clear how it causes excess phosphaturia but mutations are associated with raised concentrations of circulating FGF23, probably caused by reduced inactivation of FGF23. Its only known substrate is the acidic serine aspartate-rich MEPE-associated peptide (ASARM) motif found in short integrin-binding ligand-interacting glycoprotein (SIBLING) proteins [48].

Secretion of FGF23 is controlled by a number of factors. Dentin matrix protein 1 (DMP1) (*600980) is one of a number of SIBLING proteins that promote mineralization. It is a factor secreted mainly by osteocytes and probably acts as a mechanostat to influence bone mineralization directly, but it also inhibits FGF23 secretion. Mutations in *DMP1* cause **autosomal recessive hypophosphataemic rickets type 1** (ARHP1) (#241520) because FGF23 secretion is unrestrained. Other SIBLING proteins include bone sialoprotein (BSP) (*166490), osteopontin (OPN) (*166490), dentin sialophosphoprotein (DSPP) (*125485) and matrix extracellular phosphoglycoprotein (MEPE) (*605912). All of these SIBLING proteins contain a twenty-three amino acid ASARM sequence at the amino terminus and DMP1 also has a three amino acid Arg–Gly–Asp (RGD) sequence at the carboxy terminus. This is thought to bind with integrins on the osteocyte cell surface and allows the ASARM motif to bind with PHEX.

Some of these SIBLING proteins are upregulated in certain forms of cancer and may be responsible for alterations in FGF23 secretion that causes **tumour-induced osteomalacia** (TIO) by inhibiting binding of DMP1 with PHEX.

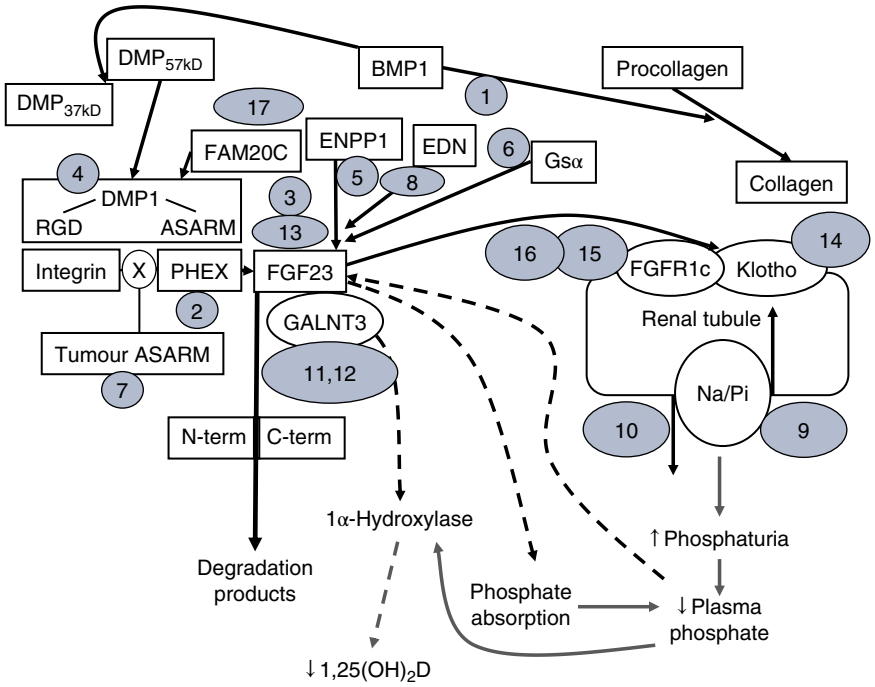


Figure 10.5 Diagrammatic representation of the 'phosphate fountain'. FGF23 is the principal factor that controls phosphate metabolism and is influenced by a host of other factors that either alter its metabolism or affect its secretion. Inhibitory effects are represented by dotted arrows and stimulation by solid arrows. Also shown in shaded circles are the conditions associated with mutations in the various genes:

1) BMP1 inactivation (osteogenesis imperfecta type XII).
2) PHEX inactivation (X-linked hypophosphataemic rickets).
3) FGF23 activation (autosomal dominant hypophosphataemic rickets).
4) DMP1 inactivation (autosomal recessive hypophosphataemic rickets type 1).
5) ENPP1 inactivation (autosomal recessive hypophosphataemic rickets type 2, also causes generalized arterial calcification of infancy).
6) GNAS somatic mutations (McCune–Albright polyostotic fibrous dysplasia).
7) MEPE somatic activation (tumour-induced osteomalacia).
8) NRAS activation (epidermal naevus syndromes, e.g. phakomatosis pigmentokeratotica, Schimmelpenning–Feuerstein–Mims, congenital melanocytic naevus syndromes).
9) NaPi2 inactivation (hereditary hypophosphataemic rickets with hypercalciuria).
10) Renal hypophosphataemia (Fanconi syndrome, various causes).
11) GALNT3 activation (hyperphosphataemic tumoural calcinosis type 1).
12) GALNT3 activation (hyperphosphataemia hyperostosis syndrome).
13) FGF23 inactivation (hyperphosphataemic tumoural calcinosis type 2).
14) Klotho inactivation (hyperphosphataemic tumoural calcinosis type 3).
15) FGFR1c inactivation (Hartsfield syndrome).
16) FGFR1c activation (osteoglophonic dysplasia).
17) FAM20C inactivation (Raine syndrome).

Some individuals with **McCune–Albright polyostotic fibrous dysplasia**, caused by somatic mutations in the alpha subunit of the *GNAS1*, have an associated excess phosphate excretion secondary to increased FGF23 by an, as yet, ill-understood mechanism. DMP1 is itself activated by cleavage by bone morphogenetic protein 1 (BMP1), a metalloproteinase that also cleaves several other proteins including procollagen type 1 [49]. Mutations in *BMP1* cause autosomal recessive **osteogenesis imperfecta type XIII** (OI13) (#614856) [50], which has a high bone mass phenotype. Mutations in type 1 collagen at the C-terminal procollagen binding site inhibit BMP1 activity and give rise to a form of autosomal dominant **OI with a high bone mass** phenotype [51].

The SIBLING proteins are highly phosphorylated by, among others, FAM20C (*611061). The latter also phosphorylates FGF23 at position 180, which prevents O-glycosylation by GALNT3 leading to increased FGF23 degradation. Mutations in *FAM20C* therefore not only inhibit the activity of the SIBLING proteins but also reduce FGF23. The net effect is to produce a bone sclerotic condition with intracerebral calcification, **Raine syndrome** (#259775) [52], which is lethal in the majority of cases although rare cases of survival into childhood are

recorded [53]. Paradoxically, it can occasionally be associated with a phosphate-wasting condition similar to XLH because of elevated FGF23.

Hypophosphataemia and rickets are seen in several primary renal tubular abnormalities, such as the **Fanconi syndrome** (whatever the cause) and in **hereditary hypophosphataemia with hypercalciuria** (HHRH) (#241530), which results from a mutation in the type 2c Na/Pi (*SLC434A3*) (*609826) co-transporter. In these conditions, FGF23 is not raised and the expected increase in $1,25(OH)_2D$ with consequent hypercalciuria may occur. This can cause troublesome nephrocalcinosis.

Parathyroid Hormone-related Peptide (PTHrP)

Following the observation that some cancers are associated with hypercalcaemia with undetectable PTH, it became apparent that another factor sharing many of the properties of PTH was the cause of the hypercalcaemia. It is now known that PTHrP (+168470) is secreted by many of these tumours [54]. PTHrP is a polypeptide with considerable homology to PTH, particularly at the N-terminal end. It is secreted as a prohormone, which is cleaved into several fragments. The N-terminal fragment binds to the PTHR1 in a similar way to PTH and has similar actions.

PTHrP does not normally circulate in amounts detectable in plasma and has no significant physiological classical hormonal actions in postnatal life but it does have important paracrine effects, particularly in cartilage. It is also of importance as the factor that promotes and maintains the positive gradient of calcium across the placenta in fetal life [55]. It is also secreted by the lactating breast and may play an important part in calcium homeostasis during lactation. Women with primary hypoparathyroidism may become hypercalcaemic while breastfeeding and require a reduction in their dose of vitamin D analogue. This effect is thought to be caused by PTHrP.

Calcitonin (CT)

CT is a polypeptide hormone secreted by the C-cells of the thyroid. Embryologically, these are derived from the ultimobranchial bodies that become incorporated into the thyroid. It is secreted in response to hypercalcaemia and acts via specific receptors mainly to counteract the effects of PTH in osteoclasts. It therefore has a calcium-lowering effect, which wanes in the presence of sustained CT secretion. Secretion also occurs in response to a specific tetrapeptide sequence present on, among other molecules, glucagon. CT acts via a receptor, the gene for which is located on chromosome 7q21.

The physiological role of CT has been difficult to establish but it may play a part in modulating bone turnover and may well be of greater importance to the developing skeleton than to the mature one. In practice, CT appears to have little clinical significance except as a therapeutic agent for acute hypercalcaemia and as a tumour marker for **medullary carcinoma of the thyroid** (MCT) (#171400).

Physiology of Bone Metabolism

Bone consists of matrix, mineral and cells, which are present in different proportions in the various parts of bone. Matrix provides the protein scaffolding on which mineral is laid down. Both components are synthesized and removed by the various bone cells. Most bones are formed from a cartilage template and are called endochondral bones. Others, notably the flat bones of the skull, the clavicles and mandibles, do not have a cartilage template and develop directly from differentiation of mesenchymal cells into bone-forming cells. These are known as intramembranous bones.

Endochondral long bones consist of an epiphysis at each end of the bone, the diaphysis or shaft and the metaphysis between them. Within these sections two different areas are seen, the tubular outer section of densely calcified bone, the cortex, and an inner more loosely packed trabecular area in which the bone is constructed from a latticework of cross struts in between which the bone marrow lies. Trabecular bone is particularly prominent in the epiphyses and diaphyses as well as in the vertebral bodies. In children, a fourth component, the cartilage growth plate, is present between the epiphysis and metaphysis. Once growth ceases, this area becomes fully ossified and disappears.

In endochondral bone, growth occurs in two ways. Increase in length occurs as a result of proliferation and subsequent ossification of the growth plates, while cortical bone increases in size by a combination of bone accretion on the outer aspect and bone resorption on the inner aspect, a process known as modelling. Intramembranous bone grows as a result of proliferation of preosteoblasts, mainly at the periphery, which results in calcification. Where the bones meet in the skull, they form sutures that interdigitate with one another. In certain conditions, including osteogenesis imperfecta (OI), this calcification process is defective and gives rise to the characteristic appearance of Wormian bones, seen radiologically as a patchwork appearance of multiple areas of calcification surrounded by undermineralized bone.

The cartilaginous growth plate contains four layers within which the predominant cell type is the chondrocyte. Furthest from the metaphysis is the resting layer beneath, which is the proliferative layer. The chondrocytes then differentiate and increase in size and their division rate decreases as they enter the pre-hypertrophic layer. Under the influence of *Indian hedgehog (IHH)* (*600726), one of a family of highly conserved genes related to Drosophila 'hedgehog' genes, further differentiation

occurs as they finally enter the hypertrophic layer, which is non-mitotic. This is regulated via PTHrP through its receptor, PTHR1, before becoming apoptotic and being invaded by blood vessels and replaced with osteoblasts. Apoptosis is dependent on the presence of adequate inorganic phosphate. Hypophosphataemia is the common link between all forms of rickets [56].

Development of the chondrocytes is influenced by the actions of a series of BMPs that are influenced in turn by another highly conserved (within vertebrates) protein called noggin (NOG) (*602991). Together they are responsible for joint development. NOG is an inhibitor of BMPs and a number of chondrodysplasias are caused by mutations in the genes for the BMPs. Mutations in *NOG* are responsible for a variety of syndromes, mostly related to abnormal joint development such as symphangylism and brachydactyly.

Bone Matrix

Collagen

The collagens are a group of proteins widely distributed in connective tissue and consist of hetero- or homotrimers of fibrils cross-linked and wound into triple helices. Eighty percent of the protein content of bone is made up of one or other type of collagen, the most abundant form of which is type I collagen. This is a heterotrimer of two strands of type 1A1 and one of type 1A2. Each strand is synthesized as a pro-protein. Translation occurs from the N-terminal end but collagen chain assembly starts at the C-terminal end. The C-terminal propeptide directs chain–chain recognition and mutations in this part of the gene result in lethal OI [57] while mutations that prevent the normal cleavage of C-terminal propeptide result in a milder form of **OI with high bone mass** [51]. During the course of post-translational modification, the pro-protein peptides, procollagen type 1 C-terminal (P1CP) and procollagen type 1 N-terminal (P1NP) peptides, are cleaved and circulate in plasma. Measurement of procollagen peptides is sometimes used as a measure of bone formation during bone turnover studies. Similarly, during bone resorption, the N-terminal cross links between fibrils (NTX) are released and can be measured, usually in urine, as an indicator of bone resorption.

Chain Alignment and Helical Folding

Mutations in the *COL1A1* (+120150) and *COL1A2* (*120160) genes result in either qualitative or quantitative abnormalities in the respective proteins causing the majority of cases of OI. The process of cross linking the collagen fibres is complex and consists of a combination of hydroxylation of lysine and proline molecules within the fibrils, aldehyde formation and glycosylation. The hydroxylation processes and helical folding are facilitated by cartilage-associated protein (CRTAP) (*605497), which forms complexes with prolyl 3-hydroxylase-1 (P3H1) (*610339). Mutations in *CRTAP* cause both **OI type IIB** and **OI type VII** (#610682) while mutations in *P3H1* (*610339), which encodes LEPRE1, cause **OI type VIII** (#610915). Cyclophylin B (CYPB) (*123841) is the third component of the prolyl 3-hydroxylase complex. Mutations in its gene, *PPIB*, cause **OI type IX** (#259440).

Quality Control of Collagen Triple Helix

SERPINH1 (*613848) and FKBP10 (*607063) are important in post-translational modification of collagen and quality control of the triple helix on entry into the Golgi complex. Mutations in their genes, *SERPINH1* and *FKBP10*, cause severe **OI type X** (OI10 #613848) and **OI type XI** (OI11 #610968), respectively. FKBP10 (*607063) and PLOD2 (*601865) are important in hydroxylation and cross linkage of collagen. Mutations in *FKBP10* and *PLOD2* cause **Bruck syndrome type 1** (#259450) and **Bruck syndrome type 2** (#609220), respectively.

Defects in Mineralization

Other genes involved in collagen formation and function include *IFITM5* (*614757) mutations in which cause **OI type V** (OI5 #610967) and *SERPINF1* (*172860) whose mutations cause **OI type VI** (OI6 #613982)

Unclassified Causes of OI

Whole exome sequencing has led to the discovery of new genes that give rise to an OI-like phenotype. These include *SEC24D* (*607186), mutations that cause **Cole-Carpenter syndrome** (CLCRT2) (#616294); *SPARC* (*182120), which encodes osteonectin and mutations that cause **OI type XVII** (OI17 #616507); *TMEM38B* (*611236), mutations that cause **OI type XIV** (OI14 #615066); *CREB3L1* (*616215), mutations that cause **OI type XVI** (OI16 #616229); *Wnt1* (*164820), mutations that cause **OI type XV** (OI15 #615220); *SP7* (*606633), mutations that cause **OI type XII** (OI12 #613849) and *PLS3* (*300131), mutations that cause **bone mineral density quantitative locus 18** (BMNDQTL18 #300910). The precise role of these genes has yet to be ascertained but they all give rise to an OI-like phenotype, some with additional clinical or radiological features that aid a definitive diagnosis.

Other Bone Collagens

The other more minor form of collagen that occurs in bone is type V (which co-localizes with type 1). It is a heterotrimer of type 5A1 (*120215), type 5A2 (*120190) and type 5A3 (*120216) collagen and mutations in one or other of these genes cause some forms of **Ehlers–Danlos** syndrome.

Four different forms of collagen are secreted by cartilage with different ones being produced at different

stages of growth plate development. During the proliferative phase, the principal form is type II (+120140) with smaller amounts of types IX (*120260) and XI (*120290). Type II collagen is also found in the vitreous of the eye and many of the conditions involving mutations of this gene are accompanied by ocular abnormalities. Once the pre-hypertrophic and hypertrophic phases are entered, type X (*120110) becomes the predominant form of collagen, which is only present in this tissue. Mutations in the genes for all these proteins can give rise to a variety of osteo- and chondrodysplasias.

Non-collagenous Matrix Proteins

The non-collagenous matrix proteins constitute the 15% or so of bone matrix not occupied by collagen. Three different types of protein are found here, proteoglycans, glycoproteins and g-carboxylated proteins (gla proteins). Proteoglycans are present either as macromolecules that fill the spaces between the collagen fibrils or as smaller proteins with more specific functions. The importance of these proteins is demonstrated by the fact that mutations in the gene for one of them, *aggrecan (ACAN)* (*155760), lead to **spondyloepiphyseal dysplasia** (#608361). These may also be associated with short stature and advanced skeletal maturation [58]. There is also a group of small leucine-rich interstitial proteoglycans whose role appears to be to bind to collagen and to growth factors.

The linking enzymes link the core proteoglycan protein to their glycosaminoglycan side chains. Mutations cause several disorders collectively known as the linkeropathies [59]. *B3GAT3* (*606373) (**multiple joint dislocations, short stature, craniofacial dysmorphism and congenital heart defects** #245600), *B4GALT7* (*615291) (**Ehlers–Danlos syndrome, progeroid type 1)** (EDSP1; #615349) and *B3GALT6* (*604327) (**Ehlers–Danlos syndrome, progeroid type 2)** (EDSP2; #130070) are the most important of these.

The glycoproteins are important components of bone and cartilage matrix since they bind to macromolecules and to cell surface receptors and help maintain cell–cell interactions. One of the most significant is cartilage oligomeric matrix protein (COMP) and mutations in the gene cause **pseudoachondroplasia** (#177170) and **multiple epiphyseal dysplasia** (#132400). The most abundant of the non-collagen proteins in calcified bone is osteonectin, a bone-specific phosphoprotein that binds selectively to hydroxyapatite and to collagen fibrils and is only found in bone and dentin. It is important in bone calcification.

The g-carboxylated (gla) proteins are vitamin K-dependent proteins important for matrix calcification and maturation. Matrix-gla protein (MGP) (*154870) seems to be important in cartilage calcification. Mutations in *MPG* cause **Keutel** syndrome (#245150) in which cartilage calcification is defective. Bone Gla protein, otherwise known as osteocalcin, is exclusively secreted by osteoblasts in proportion to their activity and is involved in bone mineral formation. Some of the osteocalcin escapes into the circulation and can be used as a measure of osteoblast activity in bone turnover studies. The relationship with vitamin K is demonstrated by the development of **chondrodysplasia punctata** in infants of mothers treated with warfarin during pregnancy.

Bone Mineral

Bone mineral consists mainly of hydroxyapatite in which ten carbon atoms are combined with three pyrophosphate molecules, each of which contains two phosphate atoms. It is laid down on the matrix scaffolding by osteoblasts under the influence of the many factors secreted by them. Pyrophosphate molecules have the structural formula

$$PO_3 - O - PO_3.$$

The bisphosphonates, which are increasingly used as a medical treatment for osteoporotic conditions, have a similar structure in which the central oxygen atom is substituted by a carbon atom, thus allowing the addition of extra residues on the two additional sidearms.

Bone Cells

Osteoblasts, osteoclasts and osteocytes exist in bone and chondrocytes in cartilage. In addition, there are those related to the haemopoietic system with which the bone cells are intimately related.

Osteoblasts

Osteoblasts are derived from mesenchymal stem cells and, under the influence of activators and inhibitors, are transformed into mature osteoblasts, the main bone-forming cells. The principal activating mechanism is via the Wnt signalling system. Wnt protein (*164820) binds to frizzled protein (Frz) (*603408) coupled to low-density lipoprotein receptor protein 5 (LRP5) (*603506) on the cell surface of the osteoblast precursors to activate the canonical pathway mediated by β-catenin [60]. This interacts with BMPs to enhance their differentiation. LRP5 is normally bound to another protein, dickkopf (*605189), which inhibits its binding to Frz. Inactivating and activating mutations of *LRP5 c*ause a variety of bone fragility conditions [61]. Inhibitors of this pathway include sclerostin (SOST) (*605740). Mutations in *SOST* cause the bone sclerosing conditions **van Buchem disease type I** (#239100) and **sclerosteosis** (#269500) (Figure 10.6).

The main function of osteoblasts is to lay down new bone, both matrix and mineral. In doing so, they secrete alkaline phosphatase and osteocalcin, both of which,

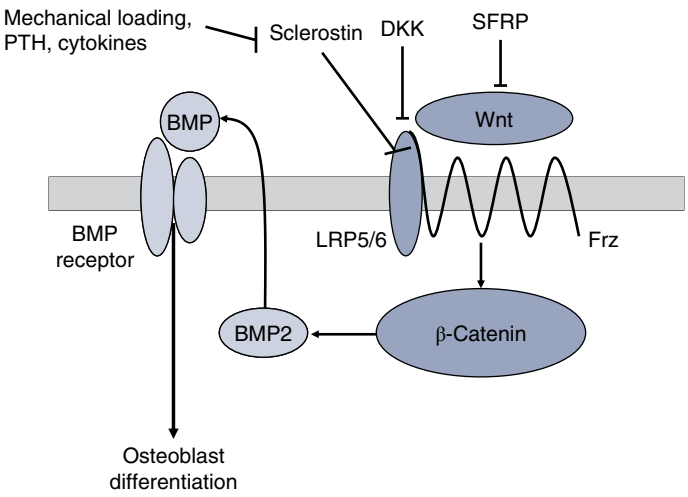


Figure 10.6 Diagrammatic representation of osteoblast differentiation. BMP, bone morphogenic protein; LRP5, low density lipoprotein receptor protein 5; DKK, Dickkopf; SFRP, soluble frizzled-related protein; Wnt, Wingless-type MMTV integration site family member; Frz: Frizzled protein. Adapted from Bodine et al. [60]. Reproduced with permission of Springer.

together with P1CP or P1NP, can be used as markers of bone turnover. They also control the activity of osteoclasts by secreting the osteoclast-transforming factors, tumour necrosis factor ligand superfamily member 11(TNFSF11) also known as rank ligand (RANKL) and tumour necrosis factor receptor superfamily member 11B (TNFRSF11B) also known as osteoprotegerin (OPG), and are themselves controlled by osteoclasts that secrete SOST.

Osteoclasts

Osteoclasts are the principal bone-resorbing cells. When they occur on the same surface of bone as osteoblasts, they act in tandem to resorb the bone, which is then replaced by the associated osteoblasts. This process is known as bone remodelling (c.f. bone modelling). Osteoclasts are derived from macrophage precursors and are under considerable influence by osteoblasts. Macrophages secrete macrophage-specific colony stimulating factor (CSF1; M-CSF), which acts on receptors (c-fms) on the osteoclast progenitors to begin the maturation process (Figure 10.7). However, M-CSF is unable by itself to complete the process that requires activation of tumour necrosis factor receptor superfamily member 11A (TNFRSF11A), also known as RANK (NF-κB), receptors on the cell surface. These are stimulated by both tumour necrosis factors (TNFs) and RANKL. Mutations in *TNFRSF11A (RANK*) cause a form of **osteoblast-poor osteopetrosis** (#612301), **early-onset Paget disease** (#602080) and **familial expansile osteolysis** (#174810).

Activation of RANK results in fusion of several cells so that the mature osteoclasts are multinucleated. RANKL is secreted by osteoblasts in response to several controlling factors including PTH, $1,25(OH)_2D$, PTHrP and various cytokines. While there are abundant PTH receptors on osteoblasts, there are very few on osteoclasts, so bone resorption has to be mediated indirectly by osteoblasts.

Osteoblasts also produce another protein, osteoprotegerin (OPG), which is a decoy receptor for RANKL. By binding to it, OPG acts as a restraining factor to prevent overactivity of RANK causing excessive bone resorption. Mutations in *TNFSF11 (RANKL)* cause a mild form of **autosomal recessive osteopetrosis** (OPTB #259710) while *TNFRSF11B (OPG)* mutations cause **juvenile Paget's disease** (#239000).

In order to resorb the bone, the osteoclast attaches itself to an area of bone by its ruffled border on its base where it forms a depression known as a resorption lacuna. The ruffled border is bounded by a skirt tightly attached to the bone and limiting the area of resorption to the base of the osteoclast. The cells are responsible for the removal of both mineral and matrix. The former is effected by the creation of an acid medium, hydrochloric acid (HCl), which dissolves the mineral. The H^+ ions are produced by carbonic anhydrase and the Cl^- ions by exchange of HCO_3^- (formed by carbonic anhydrase) for Cl^-. The protons are externalized into the resorption lacunae by an ATP-dependent vacuolar pump, T-cell immune regulator 1 (TCIRG1) (*604592), while the Cl^- ions are transported by a specific chloride channel 7 (CLCN7) (*602727). CLCN7 has a beta-subunit, osteopetrosis-associated transmembrane protein 1 (OSTM1) (*607649) while the vacuolar transport also requires pleckstrin (PLEKHM1) (*611466). Mutations in one or other of these processes impair osteoclast function and result in the various forms of **osteoclast-rich osteopetrosis**. Matrix removal requires cathepsin K (CTSK), mutations of which (*601105) cause **pycnodysostosis** (#265800) in which a high bone density phenotype is also present.

Osteocytes

Osteocytes, the most abundant bone cells, are derived from mature osteoblasts and become incorporated into bone where they are responsible for maintaining bone health. They lie in small spaces within the bone known as

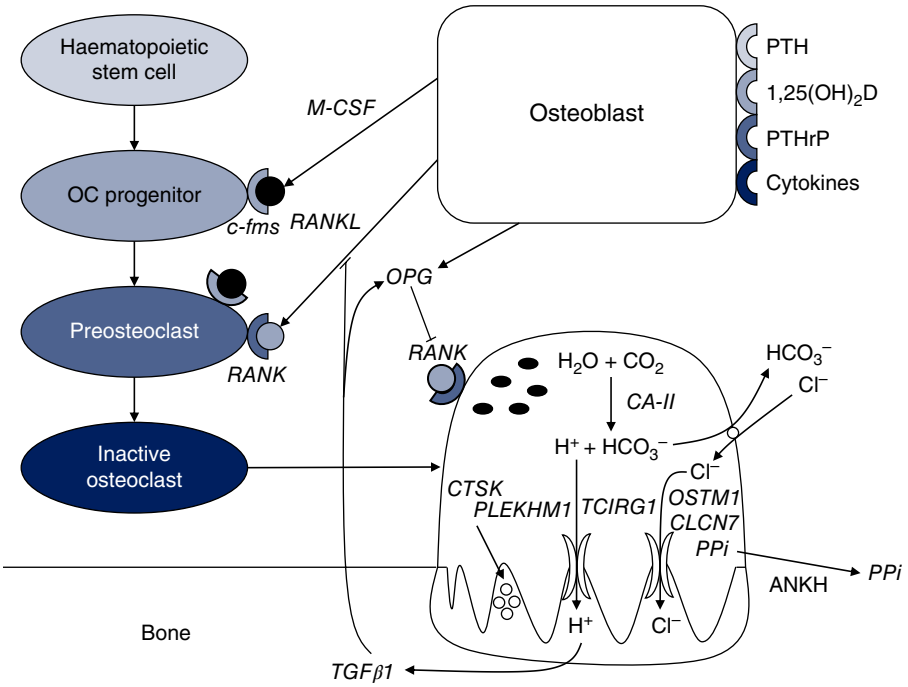


Figure 10.7 Diagrammatic representation of osteoclast differentiation and function. The upper part of the diagram shows the factors synthesized by osteoblasts that control osteoclast transformation together with the factors that stimulate osteoblasts to induce this transformation. The lower half shows an osteoclast with the various factors that they produce to maintain the acid environment that allows bone resorption. M-CSF, macrophage colony stimulating factor; PTH, parathyroid hormone; PTHrP, parathyroid hormone-related peptide; 1,25(OH)$_2$D, 1,25-dihydroxyvitamin D; PPi: pyrophosphate; ANKH, homology of mouse ANK; TCIRG1, T-cell immune regulator 1 (OPTB1); RANKL, RANK ligand (OPTB2), (PDB2, FEO); CAII, carbonic anhydrase II (OPTB3); CICN7, chloride channel 7 (OPTB4); OSTM1, osteopetrosis-associated transmembrane protein 1 (OPTB5); PLEKHM1, pleckstrin homology domain-containing protein, family M, member 1 (OPTB6); RANK, tumour necrosis factor ligand superfamily, member 11 (OPTB7); SNX10, sorting nexin-10 (OPTB8); CTSK, cathepsin K (pyknodysostosis); OPG, osteoprotegerin (juvenile Paget disease, PDB5); SOST, sclerostin (van Buchem type 1; sclerosteosis); TGFβ1, transforming growth factor, beta-1 (Camurati–Engelmann disease).

lacunae and are connected to one another by processes that are contained within canaliculae that connect the lacunae. In cortical bone these lacunae and their accompanying canaliculae are arranged in concentric circles. A group of these makes up a Haversian system.

Osteocytes maintain bone health by signalling osteoblasts and osteocytes to initiate the BMU, thereby controlling bone turnover. They are susceptible to changes in the mechanical stresses on bone via mechanostats within the cells. They also have a role to play in phosphate homeostasis by secreting FGF23 and DMP1.

Interactions between Calciotropic Agents

The aim of the interactions between the influences on calcum metabolism is to maintain the plasma-ionized calcium within narrow limits, an aim that is normally successful. At the same time, bone metabolism must be allowed to proceed so that adequate calcium and phosphate accumulation and bone remodelling can occur. The hormone factors responsible for calcium homeostasis in the face of hypocalcaemia are summarized in Figure 10.8.

Fetal and Neonatal Calcium Metabolism

Parathyroid glands are active in the human fetus from about 12 weeks of gestation maintaining a positive gradient of calcium of 0.25–0.5 mmol/L (1–2 mg/dL) across the placenta. Studies using immunoassays have demonstrated that little PTH is detectable in fetal plasma, whereas bioassays showed significant bioactivity [55]. The principal factor responsible for this bioactivity is PTHrP rather than PTH itself.

The full-term infant contains ~27 g of calcium, most of it acquired during the last trimester; the net transfer of calcium across the placenta is 300–400 mg/day at term. Turnover of calcium at birth amounts to more than 1% per day of total body calcium, compared with about 1/50th of this rate in adults. Fetal bone is therefore very active.

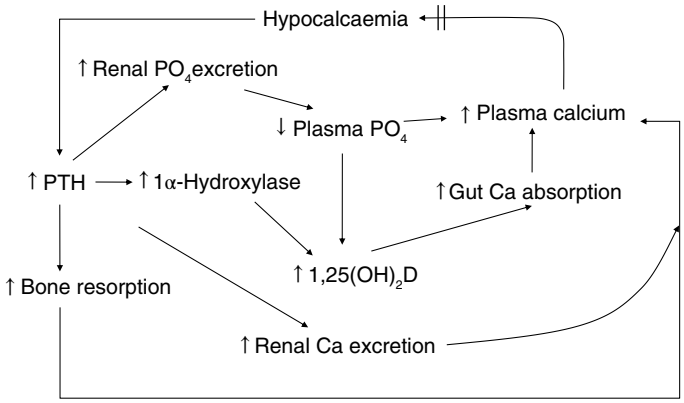


Figure 10.8 Diagrammatic representation of the principal responses to a hypocalcaemic stimulus.

Following birth, the supply of calcium from the mother ends, which results in a rapid fall in plasma calcium to a nadir of 1.8–2.0 mmol/L (7.2–8 mg/dL) in forty-eight hours. Normal concentrations (2.2–2.6 mmol/L, 8.8–10.4 mg/dL) are achieved towards the end of the first week as the supply of calcium is resumed in milk and the normal physiological mechanisms are established.

Post-Neonatal Calcium, Phosphate and Magnesium Metabolism and the Calcium Cascade

Following the establishment of normal physiological concentrations of calcium in plasma, there is little variation throughout life, despite a 50-fold increase in bone mineral to about 1200 g by adulthood. Concentrations of phosphate, however, vary considerably, being highest during periods of greatest demand for bone mineral, particularly during the neonatal period and adolescence. Concentrations of magnesium (0.7–1.2 mmol/L, 1.7–2.8 mg/dL) are maintained with little variation. Four factors, PTH, vitamin D and its metabolites, PTHrP and CT, maintain calcium homeostasis. The first two are the most important outside fetal life. Magnesium has an important part to play as deficiency interferes with PTH secretion (Figure 10.9).

Investigation of Mineral Disorders

Laboratory and Radiological Investigations

First-line investigations include measurement of total (and, if available, ionized) calcium, phosphate, albumin, magnesium, alkaline phosphatase, creatinine, PTH and 25OHD in blood, a sample of which should also be stored for future measurement of $1,25(OH)_2D$ if this is relevant, particularly if rickets is also present (Table 10.1). Paired urine should be taken for measurement of calcium, phosphate and creatinine. Fasting phosphate concentrations are of value if hypophosphataemic rickets is suspected. X-rays will reveal the presence of rickets, skeletal dysplasias (e.g. in pseudohypoparathyroidism), hyperparathyroid bone disease or soft tissue calcification. They are insensitive in detecting intracranial calcification, for which CT scanning is appropriate. Early nephrocalcinosis can best be detected by ultrasound. Where a genetic cause for a disorder is suspected, blood should be taken and DNA extracted for analysis.

Measurement of bone profile in the parents may be appropriate if maternal vitamin D deficiency or a dominantly inherited calcium disorder is suspected. It may also be appropriate to measure vitamin D concentrations in other children in the family.

Clinical Conditions

Bone Disease of Prematurity (BDP)

Most of the calcium and phosphorus acquired by the fetus are accumulated during the last trimester. Thus, if an infant is born prematurely, there has been insufficient time for bone mineralization and extremely premature infants are at risk of developing **bone disease of prematurity** (BDP). The aetiology, treatment and prevention of BDP have been reviewed [62, 63]. It is difficult to maintain mineral accretion at the same rate as occurs *in utero*, even if the infant has few neonatal problems. Complications of prematurity, such as poor nutrition, respiratory distress and cardiac problems requiring diuretic therapy, complicate the issue.

BDP has its origin in mineral, particularly phosphate, deficiency. Physical inactivity may also be a risk factor. Infants need to be monitored and nutritional supplements given as necessary. Plasma phosphate should be maintained above 1.87 mmol/L (6.4 mg/dL) and a rise in alkaline phosphatase above 500 IU/L is usually indicative of active bone disease [62]. Vitamin D supplements are usually given as a matter of routine and vitamin D deficiency

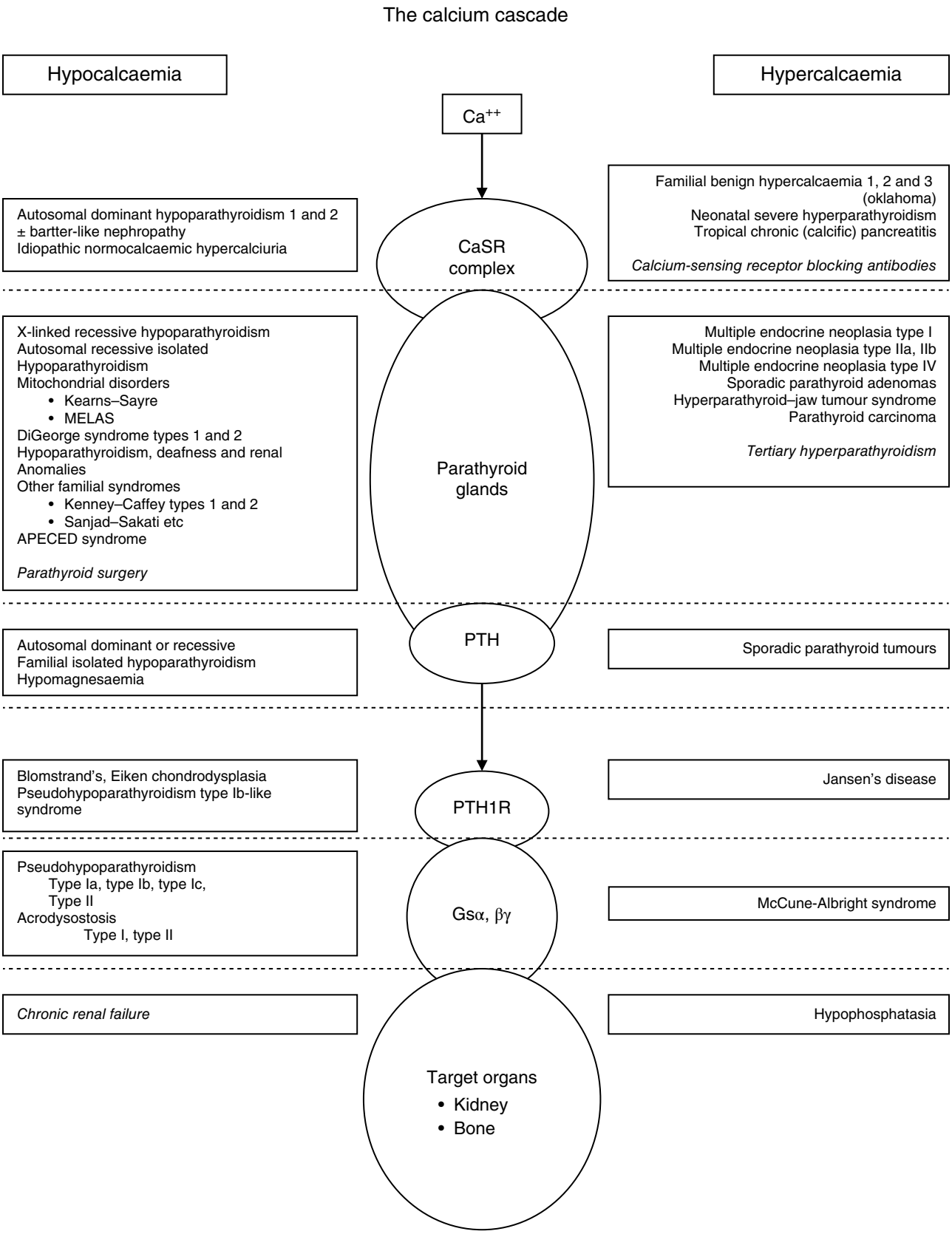


Figure 10.9 Diagrammatic representation of the calcium cascade showing the various components and the points along the cascade at which abnormalities can occur. Genetic conditions are shown in normal type and acquired conditions in italics.

Table 10.1 Investigation of disorders of calcium and bone metabolism.

Blood – Initial investigations
Calcium
Phosphate
Albumin
Alkaline phosphatase
Creatinine
25OHD
Intact PTH
Save serum for $1\alpha,25(OH)_2D$
Subsequent investigations as necessary
Blood gases $1\alpha,25(OH)_2D$
DNA analysis for genetic abnormalities
PTHrP
Bone turnover markers
Radiology and nuclear medicine
Hand and knee for rickets
Skeletal survey for bone abnormalities
Renal ultrasound for nephrocalcinosis
Parathyroid ultrasound for parathyroid tumours
CT scan for intracranial calcification
SestaMIBI scan for PT gland localization
Dexa scan
Urine – Initial investigations
Calcium
Phosphate
Creatinine
Calculate:
Ca/creat ratio
FE_{PO4} and TRP
T_mPO_4/GFR
Subsequent investigations as necessary
Glucose and amino acids
Bone turnover markers
Bone biopsy
Phosphoethanolamine, pyridoxal phosphate (vit B_6) (if hypophosphatasia suspected)
FGF23

is not usually a problem but, if neonatal hepatitis supervenes, it may be necessary to use calcitriol in addition to the other supplements if bone healing is not achieved. Other aspects of nutrition, such as protein and calorie intake, are optimized.

Hypocalcaemia

Although the plasma concentration of ionized calcium is normally maintained within narrow limits, symptoms of hypocalcaemia do not usually occur until they fall significantly. Symptoms are unusual until total calcium falls below 1.8 mmol/L (7.2 mg/dL) and some patients remain asymptomatic with a plasma calcium as low as 1.2 mmol/L (4.8 mg/dL). The rate of fall may be important in determining when symptoms occur.

Signs and Symptoms of Hypocalcaemia

Symptoms include muscle twitching and spasms, which can be painful, apnoea, stridor, carpopedal spasms and focal or generalized seizures. Measurement of plasma calcium should always be part of the investigation of unexplained fits to avoid confusion with epilepsy, even if fever is present and febrile convulsions suspected. Clinical examination may reveal positive Chvostek or Trousseau signs and chronic hypocalcaemia may cause calcification of the lens of the eye [9]. In infants whose hypocalcaemia is secondary to $1,25(OH)_2D$ vitamin D deficiency, a form of dilated cardiomyopathy may develop [64]. This does not occur with other causes of hypocalcaemia (e.g. hypoparathyroidism) and is probably the result of a direct effect of the vitamin D deficiency on cardiac muscle. If initial cardio-respiratory support is effective, the prognosis for the cardiomyopathy is good, although it may take several months to recover completely. Some syndromes are associated with dysmorphic features (e.g. 22qDS). Signs of rickets may be present. Soft tissue calcification sometimes occurs in association with pseudohypoparathyroidism, and CT scanning of the brain may reveal basal ganglia and frontal lobe calcification (Figure 10.10). The acute management of hypocalcaemia is shown in Flowchart 10.1.

Neonatal Hypocalcaemia

Hypocalcaemia may occur early in the neonatal period (within the first 2–3 days) or later (towards the end of the first week). In the former, the physiological fall in plasma calcium is exaggerated, especially in the preterm infant, following birth asphyxia, in 'sick' infants and in those born to mothers with diabetes. The mechanisms for neonatal hypocalcaemia are unclear but may represent a delayed response to the rise of PTH following hypocalcaemia. There may be an exaggerated response of CT, especially where hypoglycaemia is present because of the secretagogue effect of glucagon. This is unlikely to be the explanation in the infant of a diabetic mother since glucagon responses to hypoglycaemia are known to be impaired, presumably because of chronic hyperglycaemia *in utero*. Magnesium deficiency may be a factor, particularly

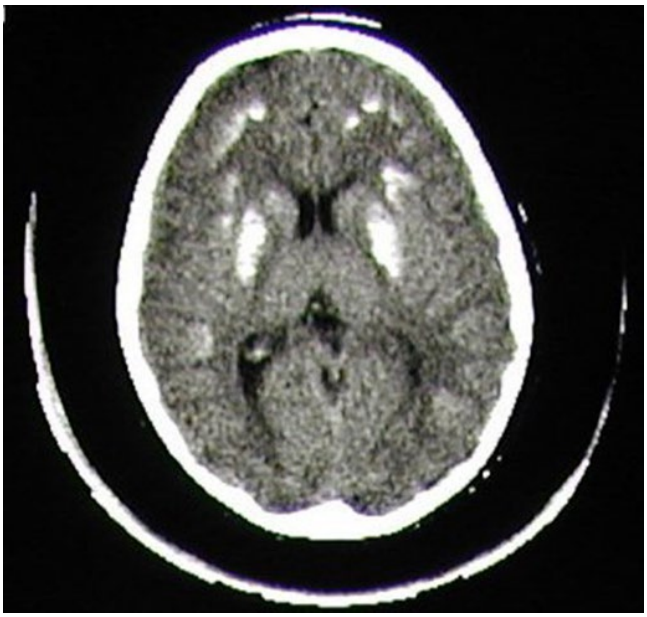
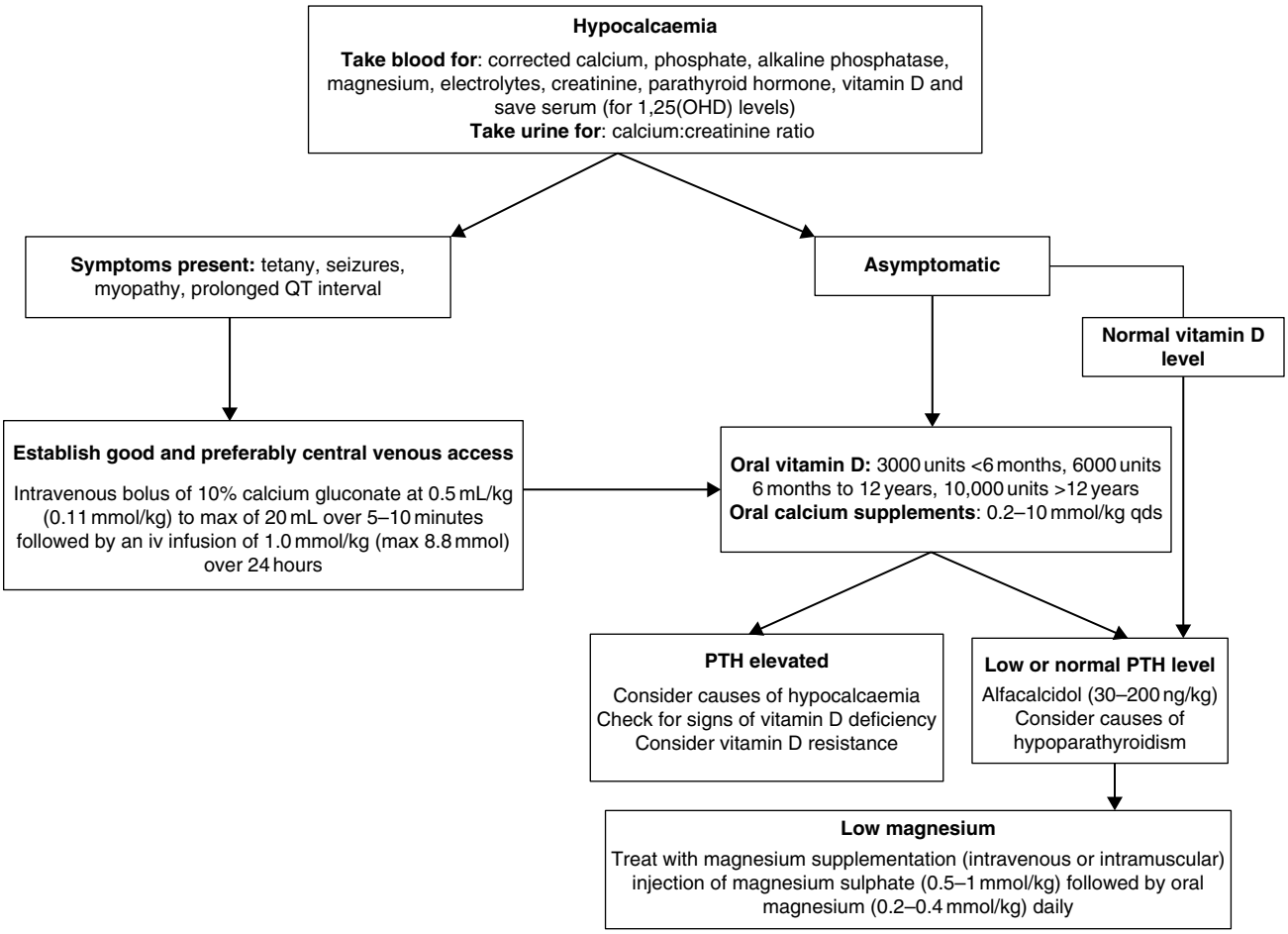


Figure 10.10 Computed tomography image of basal ganglia and frontal lobe calcification in pseudohypoparathyroidism type Ia. Extensive calcification is seen in the basal ganglia and frontal lobes.



Flowchart 10.1 Acute management of hypocalcaemia in children.

if the mother's diabetes has been poorly controlled and measurement of magnesium should be included in the investigation and corrected if necessary. Early-onset hypocalcaemia usually corrects itself spontaneously within the first week but calcium supplements are required if symptoms persist. Relative hypophosphataemia is frequently present in preterm infants and may contribute to the development of BDP.

Late neonatal hypocalcaemia is usually symptomatic and can be the first manifestation of hypoparathyroidism, but vitamin D deficiency or primary hyperparathyroidism in the mother must be considered. Hypocalcaemia due to maternal vitamin D deficiency can present at any time depending on the severity of the deficiency and is not necessarily associated with radiological evidence of rickets, particularly if the presentation is soon after birth. It is almost entirely confined to infants of mothers from ethnic minority groups and routine vitamin D supplementation of 400 IU/day, even from birth, may not be sufficient to prevent neonatal hypocalcaemia. Hyperparathyroid mothers may be asymptomatic and the presence of hypocalcaemia in the infant may be a clue to maternal disease. Measurement of vitamin D in the mother and infant and of the bone profile of the mother should form part of the investigation of late neonatal hypocalcaemia. Excess phosphate in the diet may also cause hypocalcaemia. This was attributed to the early introduction of unsuitable milk preparations and is not seen in breast fed infants nor with the increasing sophistication of formula feeds.

Symptomatic neonatal hypocalcaemia requires intravenous 10% calcium gluconate (0.225 mmol/mL, 0.9 mg/mL) given as a slow infusion of 1–3 mL/kg that can be continued as an infusion of 1–2 mmol/kg/day (40–80 mg/kg/day) or as oral supplements. It is important to ensure that the infusion is given into a secure intravenous site because extravasation causes unsightly burns. In the event of vitamin D deficiency, additional vitamin D supplements of 1000–1500 IU/day are required. If hypocalcaemia persists, and particularly if hypoparathyroidism is suspected, active metabolites of vitamin D may be required.

Decreased Release of PTH

Disorders of the Parathyroid (PT) Glands

Isolated Congenital Hypoparathyroidism

This can be sporadic or inherited as autosomal dominant, autosomal recessive or X-linked recessive, leading to abnormal development of PT glands; the genes involved with isolated hypoparathyroidism include *CaSR*, *GNA11*, *PTH*, *GCM2* and *SOX3*.

The most common cause of isolated congenital hypoparathyroidism (#146200) is due to a mutation of the *glial cell missing 2* gene (*GCM2* – also referred to as *GCMB*) (*159623) on chromosome 6p24.2, which is a highly conserved transcription factor responsible for PT gland development. *GCM2* has also been shown to regulate the expression of the *CaSR* and *PTH* genes. This mutation can be transmitted both as autosomal recessive [33, 65] and dominant forms [66].

Mutations in the *preproPTH* gene on chromosome 11p15 (*168450) lead to disruption of processing and translocation of PTH across the endoplasmic reticulum and the cell membrane for exocytosis resulting in cell apoptosis. These mutations have been described in kindreds with autosomal dominant [67] and recessive transmission [68]. Genetic variants on chromosome Xq26-q27, probably associated with *SOX3* dysregulation, (*313430) give rise to an **X-linked recessive early-onset hypoparathyroidism with severe hypocalcaemia and related seizures** (#300123) [69].

Hypoparathyroidism Related to Syndromes

The **Kearns-Sayre syndrome** (KSS) (#530000) comprises hypoparathyroidism with progressive external ophthalmoplegia, pigmentary retinopathy, heart block or cardiomyopathy and proximal myopathy. It may also be associated with diabetes mellitus. It overlaps with the **MELAS** syndrome (#540000) in which hypoparathyroidism is associated with a childhood onset of mitochondrial encephalopathy, lactic acidosis and stroke-like episodes [9]. Proximal myopathy and diabetes mellitus have also been described with this condition. Several different maternally transmitted mutations in the mitochondrial genome have been reported in some of these patients, although the role of these mutations is not well understood.

The **22q deletion syndome** (22qDS previously known as CATCH 22) is the commonest gene deletion syndrome (*602054) in man with an incidence of 1 in 4000–5000 live births. It consists of a tetrad of PT gland hypoplasia, thymic immunodeficiency, congenital heart disease and facial anomalies, structures all derived from the third and fourth branchial pouches [70]. It includes several overlapping conditions, including the **DiGeorge** (#188400) (DGS), **velocardiofacial** (VCFS) (#192430) and **conotruncal anomaly facial** (CTAFS) (#217095) syndromes, and a number of non-syndromic cardiac conditions, such as pulmonary atresia with ventricular septal defect, Fallot's tetralogy, truncus arteriosus and interrupted aortic arch. Only the DGS includes hypoparathyroidism. The very variable nature of these conditions is seen from their original clinical descriptions.

Most cases of DGS arise *de novo* and are associated with deletions of variable size in chromosome 22q11.2. Autosomal dominant transmission has been described in association with an unbalanced translocation and

deletion involving the same chromosomal area [71]. *TBX1* (*602054), which is in the centre of the DiGeorge region of chromosome 22q11.1, seems to play a crucial role in the early development of the pharyngeal pouches and otic vesicles. The precise role of the gene products is not well understood but they are probably DNA-binding proteins [72]. Another gene, *UDF1L*, is also located within the 22q11 region, and deletions of it have been found in all patients with the 22q11.1 DGS syndrome [73].

Not all patients with DGS have mutations in the 22q region but those that have are designated DGS1. Mutations in a second locus on chromosome 10p13-14 have also been seen in association with hypoparathyroidism and immune deficiency, which has been designated DGS2 (%601362). The gene involved in this syndrome is not known. Some of the features of DGS may be seen in **fetal alcohol syndrome**.

In DGS, the emphasis is on the PT and thymus glands and the cardiac anomalies. The severity of the condition varies but most infants with this syndrome present with cardiac abnormalities, which may require urgent attention. Developing hypocalcaemia does not become immediately apparent and is frequently overlooked. Thymus gland aplasia is suspected by the absence of a thymic shadow on chest X-ray and can be confirmed by a low T-cell count, although the total lymphocyte count may be normal. Late-onset DGS has also been described. These patients present with hypocalcaemia in late childhood or adolescence and have only minor dysmorphic features. Microdeletions of the 22q11 chromosome have also been identified in them [74]. If hypocalcaemia does not become symptomatic during infancy, it may remain 'dormant' until adolescence when the increased demand for calcium during the adolescent growth spurt precipitates hypocalcaemic convulsions. The use of loop diuretics for cardiac failure may precipitate hypocalcaemia because of their hypercalciuric effects. For a comprehensive review of 22qDS, see [75] and [76].

In autosomal dominant **hypoparathyroidism, deafness and renal anomalies** (HDR) (#146255**)** hypoparathyroidism is associated with low or inappropriately normal PTH with normal responsiveness to PTH [77]. Deafness is sensorineural and the renal anomalies consist of cystic changes that lead to renal impairment in some patients. Cytogenetic abnormalities of chromosome 10p14-10pter have been identified in these patients. This region does not overlap the DGS2 region and contains a gene, *GATA-binding protein 3* (*GATA3)*, (*131320) that is involved in the developing kidney, otic vesicles and PT glands.

The autosomal recessive **Kenny-Caffey** (#244460) and **Sanjad-Sakati** [78] syndromes as well as that described by Richardson and Kirk [79] are probably all variants of the same condition known as the **hypoparathyroidism retardation dysmorphism** (HRD) syndrome (#241410). They are all caused by mutations in the *tubulin-specific chaperone E (TBCE)* gene (*604934) and, despite phenotypic differences, appear to share a common haplotype. Hypoparathyroidism is associated with extreme short stature and developmental delay. They have been described mainly in consanguineous families from Saudi Arabia and Kuwait and mutations have been mapped to chromosome 1q42-43. Other familial syndromes are also described, although the chromosomal locations and gene defects have not been identified (Table 10.2).

The APECED syndrome (#240300), also known as the **polyglandular autoimmune type 1 syndrome**, is an evolving association between mucocutaneous candidiasis and hypoparathyroidism that usually develops in mid-childhood [80]. About 70% of patients develop adrenal insufficiency and other endocrinopathies, such as hypogonadism and hypothyroidism; diabetes mellitus may develop in later life. Other associated features include nail pitting, keratopathy, alopecia, hepatitis and intestinal malabsorption.

Several mutations in the *autoimmune regulator 1 (AIRE-1)* gene (*607358), which is located on chromosome 21q22.3, have been recognized. Its role in regulating immune function is not known but it is a 545-amino acid protein that probably acts as a transcriptional factor and is located mainly in the nucleus. The condition is particularly prominent in Finnish families, in which a mutation at codon 257 (Arg257Ter) has been identified in 82% of subjects. It has also been identified in Iranian Jews.

Patients usually present with mucocutaneous candidiasis and later develop hypoparathyroidism followed by other features. Adrenal insufficiency should be suspected if hypercalcaemia occurs in a previously stable patient. This is probably because of changes in renal calcium reabsorption following the hypovolaemia associated with mineralocorticoid or glucocorticoid deficiency.

Disorders of Calcium Sensing

Autosomal Dominant Hypocalcaemia (ADH)

This condition alters the CaSR in the PT glands and kidneys causing a lower threshold for maintaining the serum calcium. The biochemical profile comprises hypocalcaemia, hypercalciuria and an inappropriately normal or low PTH. Symptoms of hypocalcaemia are dependent on the severity of serum hypocalcaemia and nephrocalcinosis is a common complication.

ADH is caused by a gain-of-function (activating) mutation of *CASR* (*119185) (**ADH1**) (#601199) or *GNA11* (**ADH2**) (#139313), which encodes the G-protein subunit involved in CaSR signalling. Calcium is sensed as being 'normal' at subphysiological concentrations and PTH secretion is therefore switched off inappropriately, causing

Table 10.2 Hypocalcaemic disorders associated with genetic abnormalities. These disorders are shown according to their location in the calcium cascade. The metabolic abnormalities, together with the OMIM numbers of the conditions and their genes, modes of inheritance and principal clinical features are shown.

Location in calcium cascade	Metabolic abnormality	OMIM	Location	Gene	Gene product	OMIM	Inheritance	Principal clinical features
Calcium sensing receptor								
	Autosomal dominant hypocalcaemia type 1 (familial-isolated hypoparathyroidism)	#601198	3q13.3–q21	*CaSR*	Calcium sensing receptor	*601199	AD	(Symptomatic) hypocalcaemia, hypercalciuria, nephrocalcinosis
	Autosomal dominant hypocalcaemia with Bartter-like features	#601198	3q13–21	*CaSR*	Calcium sensing receptor	*601199	AD	(Symptomatic) hypocalcaemia, hypercalciuria, nephrocalcinosis
	Autosomal dominant hypocalcaemia type 2	#615361	1pter-p36.13	*GNA11*	Guanine nucleotide-binding protein Alpha 11	*139313	AD	(Symptomatic) hypocalcaemia, hypercalciuria, nephrocalcinosis
The parathyroid glands								
	X-linked recessive hypoparathyroidism	%307700	Xq26–27	*?SOX3*	SRY-related homeobox	*313430	XLR	Infantile onset hypoparathyroidism
	Autosomal recessive isolated hypoparathyroidism	#146200	6p24.2	*GCMB*	Homologue of drosophila glial cells missing	*603716	AR and AD	Isolated hypoparathyroidism
	Mitochondrial disorders							
	Kearns-Sayre	#530000	Mitochondrial gene deletion	Various mitochondrial			Maternal	Hypoparathyroidism, progressive ophthalmoplegia, pigmentary retinopathy, heart block or cardiomyopathy, short stature, primary gonadal failure, sensorineural deafness, proximal myopathy, diabetes mellitus
	MELAS	#540000	Mitochondrial gene point mutation	Various mitochondrial			Maternal	Hypoparathyroidism, mitochondrial encephalopathy, lactic acidosis, stroke-like episodes, proximal myopathy, diabetes mellitus
	Pearson marrow pancreas	#557000	Mitochondrial contiguous gene deletion	Various mitochondria			Maternal	Hypoparathyroidism, sideroblastic anaemia, pancreatic dysfunction
	DiGeorge syndrome – type I	#188400	22q11.2	*TBX1* (and others)	Transcription factors	*602054	Sporadic or AD or unbalanced translocation	Neonatal hypoparathyroidism, thymic aplasia, ear, nose and mouth deformities, aortic arch abnormalities – truncus arteriosus, right-sided aortic arch, etc.

DiGeorge syndrome – type II	#188400	10p13-14	?	?		Sporadic	Neonatal hypoparathyroidism, immune deficiency
Hypoparathyroidism, deafness, renal anomalies	#146255	10p14-10pter	*GATA3*	T-cell antigen receptor enhancer binding protein	*131320	AD	Hypoparathyroidism, sensorineural deafness, cystic renal changes
Other familial syndromes							
Autosomal dominant Kenny-Caffey	#127000	11q21.1	*FAM111A*	Family with sequence similarity 111A	*615292	AD	Similar to AR Kenney-Caffey
Gracile bone dysplasia	#602361	11q21.1	*FAM111A*	Family with sequence similarity 111A	*615292	AD	Perinatal lethal condition. Thin dense metaphyses, flared metaphyses, brachydactyly, ocular abnormalities
Autosomal recessive Kenny-Caffey	#244460	1q43-44	*TBCE*	Tubulin-specific chaperone E	*604934	AR	Hypoparathyroidism, extreme short stature, cortical thickening and medullary stenosis of tubular bones, normal bone age, absent diploic space, delayed closure of anterior fontanelle, normal intelligence
Sanjad-Sakati and Richardson and Kirk	#241410	1q43-44	*TBCE*	Tubulin-specific chaperone E	*604934	AR	Hypoparathyroidism, deep set eyes, microcephaly, thin lips, long philtrum, beaked nose, external ear anomalies, micrognathia, depressed nasal bridge, mental retardation
Dahlborg and Borer		?	?	?		AR or XLR	Hypoparathyroidism, congenital lymphoedema, nephropathy, mitral valve prolapse, brachytelephalangy
Autoimmune polyglandular syndrome type 1 (APECED1)	#240300	21q22.3	*AIRE-1*	Autoimmune regulator	*607358	AR	Mucocutaneous candidiasis, hypoparathyroidism, adrenal insufficiency, hypogonadism, diabetes mellitus, nail pitting, keratopathy, alopecia, hepatitis, intestinal malabsorption
Autoimmune polyglandular syndrome type 2 (Schmidt) (APS2)	%269200	?	?			AD	Hypoparathyroidism, Addison's disease, type 1 diabetes
Long chain hydroxyacyl CoA dehydrogenase deficiency/ trifunctional protein deficiency	#609015	2p23.3	*HADHA/B*	Trifunctional protein	*600890/ *143450	AR	Hypoparathyroidism, multisystem metabolic disorder. Neonatal lethal, infantile Reye-like syndrome or adolescent skeletal myopathy

(*Continued*)

Table 10.2 (Continued)

Location in calcium cascade	Metabolic abnormality	OMIM	Location	Gene	Gene product	OMIM	Inheritance	Principal clinical features
PTH								
	Familial-isolated hypoparathyroidism	#146200	11p15	*PTH*	Parathyroid hormone	*168450	AD	Hypoparathyroidism
	Familial-isolated hypoparathyroidism	#146200	11p15	*PTH*	Parathyroid hormone	*168450	AR	Hypoparathyroidism
PTH/PTHrP Receptor								
	Blomstrand's chondrodysplasia	#215045	3p21.1-p22	*PTH1R*	Parathyroid hormone receptor	*168468	AR	Advanced bone maturation, accelerated chondrocyte maturation, increased bone density, poor bone modelling, rapidly lethal
	Eiken skeletal dysplasia	#600002	3p21.1-p22	*PTH1R*	Parathyroid hormone receptor	*168468	?AR	Retarded ossification. Abnormal bone modelling.
	Pseudohypoparathyroidism type Ib – like syndrome		3p21.1-p22	*PTH1R*	Parathyroid hormone receptor	*168468	?AR	Hypoparathyroidism with raised PTH
Post Receptor Events								
	Pseudohypoparathyroidism type Ia	#103580	20q13.2-13.3	*GSαAD* paternally imprinted	Gs-alfa subunit	+139320	AD paternally imprinted	Hypoparathyroidism with raised PTH, short stature, round facies, short metacarpals and metatarsals (Albright's hereditary osteodystrophy), mild hypothyroidism, disturbance of ovarian function, mild developmental delay
	Pseudopseudohypoparathyroidism		20q13.2–13.3	*GSαAD* maternally imprinted	Gs-alfa subunit	+139320	AD maternally imprinted	As above but with no hypoparathyroidism
	Pseudohypoparathyroidism with testotoxicosis		20q13.2–13.3	*Gsα* – differential heat sensitivity	Gs-alfa subunit	+139320	AD paternally imprinted	As for PHP-Ia but with testotoxicosis
	Pseudohypoparathyroidism type Ib	#603233	20q13	*STX16*	Syntaxin 16	*603666	AD ?paternally imprinted	Hypoparathyroidism with raised PTH but no features of AHO. May retain bone sensitivity
	Pseudohypoparathyroidism type Ic		20q13	*GNAS*			AD	Multiple hormone resistance with AHO
	Pseudohypoparathyroidism type II	%203330	?	?	?		?	Hypoparathyroidism with normal cAMP but impaired phosphaturic response
	Acrodysostosis type 1	#101800	17q24.2	*PRKAR1A*	Regulatory camp subunit	*188830	AD	Acrodysostosis, variable hormone resistance
	Acrodysostosis type 2	#614613	5q11.2-q12.1	*PDE4D*	Phosphodiesterase type 4D	*600129	AD	Acrodysostosis, rarely hormone resistance

Magnesium Deficiency

Familial primary hypomagnesaemia	#602014	9q12–22.2	*TRPM6*	Transient receptor potential cation channel M6	*607009	AR	Isolated defect of magnesium transport in the gut, hypocalciuria
Isolated renal magnesium wasting	#154020	11q23	*FXYD2*	Na,K-ATPase γ	*601814	AD	Hypermagnesuria with hypomagnesaemia. Hypocalciuira.
Isolated recessive renal hypomagnesemia	#611718	4q25	*EGF1*	Epidermal growth factor	*131530	AR	Isolated hypomagnesemia, normal plasma and urine calcium, psychomotor retardation, seizures, brisk reflexes
Gitelman's syndrome	#263800	16q13	*SLC12A3*	Thiazide-sensitive Na-Cl cotransporter	*600968	AR	Hypermagnesuria, hypokalaemic alkalosis, hypocalciuria, chronic dermatitis
Familial hypomagnesaemia with hypercalciuria and nephrocalcinosis	#248250	3q	*CLDN16 (PCLN-1)*	Paracellin-1	*603959	AR	Hypermagnesuria, hypercalciuria, hypomagnesaemia, nephrocalcinosis, renal failure
Renal hypomagnesemia with ocular involvement	#248190	1p34.2	*CLDN 19*	Claudin 19	*610036	AR	Hypermagnesuria, hypercalciuria, hypomagnesaemia, nephrocalcinosis, renal failure, ocular abnormalities
Hypomagnesaemia type 6	#613882	10q24.32	*CNNM2*	Cyclin divalent metal cation transporter	*607803	AD	Renal hypomagnesaemia
Hypomagnesaemia with episodic ataxia	#160120	12p13.32	*KCNA1*	K channel voltage gated	*176260	AD	Hypomagnesaemia with episodic ataxia
Bartter type 1	#601678	15q21.1	*SLC12A3*	Sodium-potassium-chloride cotransporter-2	*600839	AR	Hypokalaemic hypochloraemia alkalosis, salt wasting, hypercalciuria, nephrocalcinosis, osteopenia
Bartter type 2	#241200	11q24.3	*KCNJ1*	Inward-rectifying apical potassium channel	*600359	AR	Hypokalaemic hypochloraemia alkalosis, salt wasting, hypercalciuria, nephrocalcinosis, osteopenia
Bartter type 3	#607364	1p36.13	*CLCNKB*	Kidney chloride channel B	*602023	AR	Hypokalaemic hypochloraemia alkalosis, salt wasting, hypercalciuria, nephrocalcinosis, osteopaenia, occasional hypomagnesemia
Bartter type 4a	#602522	1p32.3	*BSND*	Barttin	*606412	AR	Hypokalaemic hypochloraemia alkalosis, salt wasting, hypercalciuria, nephrocalcinosis, osteopenia
Bartter type 4b	#613090	1p36.131	*CLCNKA/B*		*603024/3	AR	
East syndrome	#612780	1q23.2	*KCNJ10*		*602208	AR	

hypoparathyroidism. The extent of the resulting hypoparathyroidism is determined by how much the mutation shifts the calcium response curve to the left (Figure 10.2). Patients may or may not be asymptomatic. Several mutations have been described and although there is no genotype–phenotype correlation, symptoms are related to the degree of hypocalcaemia, which remains fairly constant within individuals. Inheritance is usually autosomal dominant but sporadic cases have been described.

The need for treatment depends on whether or not symptoms are present. In patients whose plasma calcium is above 1.95 mmol/L (7.8 mg/dL), treatment is unnecessary and required only with lower calcium concentrations if symptoms are present. An active vitamin D metabolite (alfacalcidol or calcitriol) should be used cautiously and in the smallest dose required to prevent symptoms. It is not necessary to restore plasma calcium to normal. Urinary calcium excretion should be monitored carefully to avoid nephrocalcinosis and regular renal ultrasonography can be helpful in detecting early changes. If it proves difficult to prevent symptomatic hypocalcaemia without causing nephrocalcinosis, thiazide diuretics may also be used. Selective CaSR blocking agents may become available in the future. Patients who prove difficult to control and who have problems with nephrocalcinosis have responded well to infusions of PTH 1-34 (teriparatide) [81].

Impairment of the CaSR in the renal tubules is associated with metabolic alkalosis, impaired renal function, hypercalciuria, nephrocalcinosis, hyper-reninaemia, hypokalaemia and hyperaldosteronism in various degrees. These children may have presented in the neonatal period with a short episode of hypoparathyoidism and then later in childhood develop a Bartter-like syndrome. In all cases, activating mutations of *CaSR* were identified resulting in a marked left shift of the dose–response curve for extracellular calcium signalling. The mechanism of these biochemical changes is not clear but it has been suggested that the CaSR defect prevents calcium and magnesium reabsorption, which normally takes place in conjunction with sodium. This leads to sodium wasting and a concentrating defect that stimulates hyper-reninaemia and hyperaldosteronism and leads to increased potassium losses. At the same time, the DCT then compensates for the sodium losses that leads to further calcium excretion and hypokalaemic alkalosis. Caution is urged in the use of thiazide diuretics, which might worsen calcium excretion.

Other acquired non-genetic forms of hypoparathyroidism include PT gland destruction during thyroid surgery or following parathyroidectomy. Isolated autoimmune hypoparathyroidism may also occur, and destruction of the PT glands can occur following iron overload with multiple transfusions in β-thalassemia major.

Raised PTH

In **pseudohypoparathyroidism (PHP)** apparent hypoparathyroidism is accompanied by elevated PTH. The defect is in the G-protein-coupled hormone receptor to PTH, which is an imprinted gene. PHP can be divided into type I, in which there is blunting of renal tubular cAMP response to PTH infusion and impaired urinary phosphate excretion, and type II, which is associated only with impaired urinary phosphate excretion.

Type I is further subdivided according to other associated features. **Type Ia (PHP-Ia)** (#103580) is an autosomal dominant condition characterized by hypocalcemia and hyperphosphatemia but with raised PTH. Resistance to PTH can be confirmed by demonstrating lack of cyclic AMP or phosphaturic responses to PTH infusion. A characteristic set of features includes short stature, round facies, shortening of the metacarpals and metatarsals, particularly the fourth and fifth, and obesity collectively termed **Albright's hereditary osteodystrophy** (AHO) (Figure 10.11). Other features include intracranial calcification (Figure 10.10), sensorineural deafness and a poor sense of smell. Resistance to other cyclic AMP-dependent hormones, especially TSH and gonadotropins, may be present, leading to mild hypothyroidism and menstrual irregularity. Following the discovery of the Gsα subunit of the G-protein (*139320), it was recognized that inactivating mutations within *GNAS1* are responsible for the PTH resistance [82].

AHO can present without an abnormality of calcium metabolism and this is called **pseudo-pseudohypoparathyroidism (PPHP)**. Both conditions can occur within the same family but not within the same sibship. While both conditions are associated with skeletal manifestations, when hypocalcaemia is present, the gene is inherited from an affected mother; paternal transmission of the gene does not result in PTH resistance, despite the fact that identical mutations could be demonstrated within families. Gene imprinting was suspected [82] and subsequently confirmed by detailed genetic studies.

GNAS1 has 13 exons that encode the Gsα subunit plus an additional 7 exons including those that encode transcripts known as A/B, XL and NESP55. By a complex arrangement of splicing, four different mRNA transcripts are known to result (Figure 10.12). All have the products of codons 2–13 in common.

Native Gsα also contains exon 1 and this mRNA is expressed in most tissues in a biallelic manner. However, the transcripts containing A/B and XL are expressed only by the paternal allele because the maternal allele is methylated, resulting in inactivation; the NESP55 allele is expressed only in the maternal allele, the paternal allele being methylated [84]. In the kidney only the maternal allele is expressed in the proximal tubule (where most

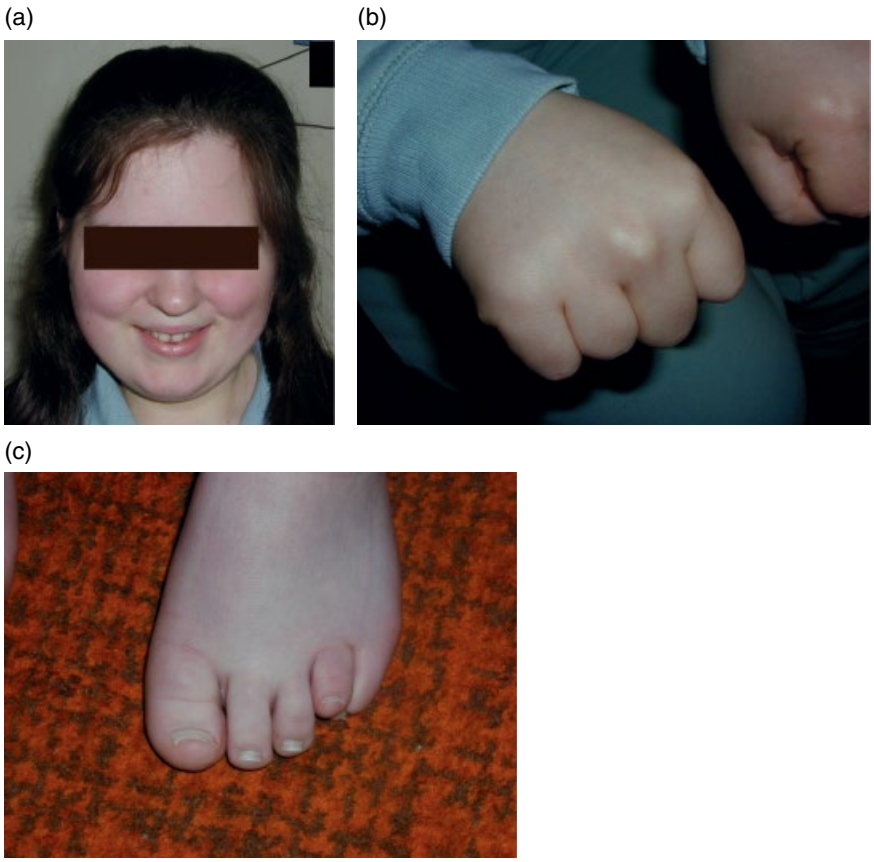


Figure 10.11 Photographs of the face (a), showing the typical rounded facies, and of the right hand (b) and left foot (c) of a patient with pseudohypoparathyroidism type Ia showing the typical shortening of the metacarpals and metatarsals seen as part of Albright's hereditary osteodystrophy (AHO). She had presented with short stature. A mutation in the GNAS1 gene has been demonstrated in this patient. *Source:* Reproduced with the kind permission of the patient. (*See insert for colour representation of the figure*.)

calcium reabsorption occurs) although it is expressed biallelically in the TALH and collecting ducts. This causes hypocalcaemia although, in contrast to hypoparathyroidism, hypercalciuria is not usually a problem during treatment because of the presence of an active paternal allele lower down the tubule. Thus, if a mutation occurs within the maternal allele, PTH resistance and hypocalcaemia are present while hypocalcaemia is absent if the paternal allele is mutated.

Heterotopic calcification occurs in haploinsufficiency with heterozygous mutations of either maternal or paternal allele but obesity arises from imprinting regions of the hypothalamus and only occurs in patients with PHP1a with maternal mutations [85].

Several mutations have been described throughout the *GNAS1* gene, but most frequently in exon 7. There is no obvious phenotype–genotype correlation, apart from a missense mutation at codon 366 consisting of an Ala122Ser substitution, which results in a temperature-sensitive Gsα mutant. At 37 °C, it is inactivated, resulting in PHP; at 34 °C, it is activated and results in a form of **testotoxicosis** [86].

PHP type Ib is associated with PHP without features of AHO. PTH resistance develops slowly and other forms of hormone resistance, although present, can be mild but are present when there is maternal inheritance and can be due to a methylation defect at the GNAS locus. Many of these cases are due to *de novo* mutations.

PHP type Ic (PHPIc) has a phenotype similar to that of PHPIa but is caused by methylation defects in the maternal allele [87] of *GNAS1*. The mutation does not affect adenylate cyclase activity but interferes with receptor-mediated activation and is thus associated with hormone resistance [88]. The defect is not present in erythrocytes, which precludes making a genetic diagnosis on peripheral blood.

PHP type II is associated with hormone resistance and a normal cAMP response to infused PTH. The underlying genetic mechanism is still unclear. These patients have hypocalcaemia and high plasma phosphate concentrations in association with an elevation in PTH. A similar clinical condition may occur in vitamin D deficiency.

Acrodysostosis

Two forms of this condition that consists of short stature, facial dysostosis and severe brachydactyly are

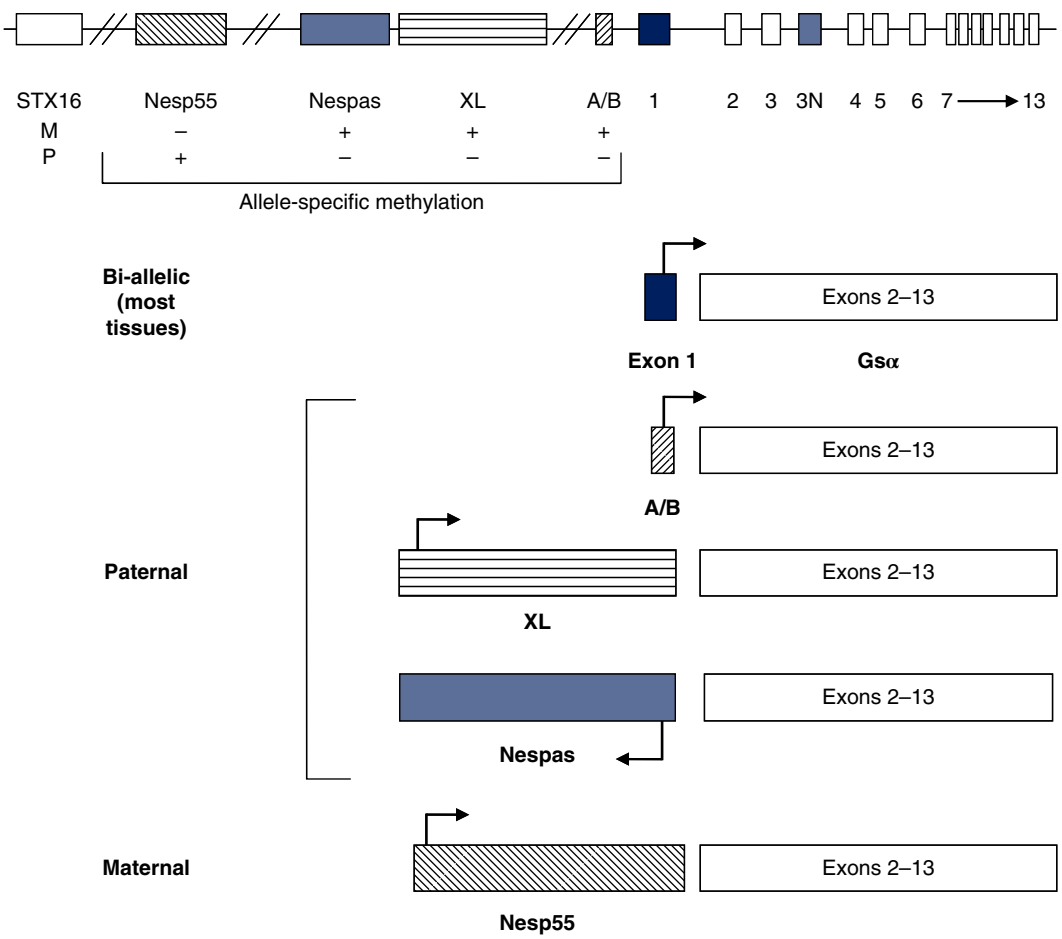


Figure 10.12 Diagrammatic representation of the intron/exon organization of the GNAS1 gene showing the different mRNAs that are derived as a result of alternative splicing. Native Gsα is thought to be expressed in most tissues and to be bi-allelic. Mutations result in AHO. The A/B, XL and nespas alternative transcripts are principally expressed in the paternal allele while the Nesp55 transcript, which is mainly found in the kidney, is expressed from the maternal allele. Therefore, paternally acquired mutations result in pseudo-pseudohypoparathyroidism while pseudohypoparathyroidism results from maternally acquired mutations. *Source:* Adapted and reprinted from Bastepe et al. [83] with permission from Elsevier.

known. The radiological appearance is that of distal phalangeal acro-osteolysis. **Acrodysostosis type 1** (ACRDYS1) (#101800) is caused by inactivating mutations in *PRKAR1A* (*188830), which prevent dissociation of the catalytic and regulator subunits of the protein kinase and is often associated with hormone resistance [89]. **ACRDYS2** (#614613) is related to activating mutations in *PDE4D* (*600129) on chromosome 5q12. This has a similar phenotype to ACRDYS1 but is less commonly associated with hormone resistance and many of these patients have spinal stenosis and learning difficulties. Different activating mutations of the regulatory subunit of *PRKAR1A* are associated with the **Carney complex type 1** in which adrenal tumours feature strongly and inactivating mutations of *PDE4D* have been described in association with adrenal hypertrophy.

Treatment of Hypoparathyroidism and Pseudohypoparathyroidism

Treatment is aimed at maintaining plasma calcium concentrations within the lower part of the normal range without causing hypercalciuria. The mainstay of treatment is vitamin D either in its active form, 1α,25$(OH)_2$D (calcitriol) or the analogue 1α-hydroxycholecalciferol (alfacalcidol). The dose of calcitriol is usually 15–30 ng/kg/day to maintain normocalcaemia but requires twice- or thrice-daily dosage. Alfacalcidol usually requires about twice the dose but, because it has to be metabolized first, it has a longer half-life and needs to be given only once daily. Calcium supplements are usually required and may enable the dose of alfacalcidol to be reduced, which is a particular advantage in hypoparathyroid disorders in which the renal tubular reabsorptive

effects of PTH are lacking and hypercalciuria may supervene. In patients in whom cardiac failure is present (e.g. DiGeorge syndrome), loop diuretics such as frusemide should be used with caution as the hypercalciuric effects of these agents may precipitate symptomatic hypocalcaemia. Regular renal ultrasound examination is useful in detecting early nephrocalcinosis.

The principles of treatment of PHP are similar to those of hypoparathyroidism. Alfacalcidol (30–50 ng/kg/day) is usually sufficient to maintain normocalcaemia. Hypercalciuria is less likely to occur than in primary hypoparathyroidism and the plasma calcium concentration can usually be kept well within the normal range. Patients with PHP1a or PHP1c, and occasionally PHP1b, may have resistance to other hormones. TSH is frequently slightly raised and thyroxine is required to suppress this and allow optimal thyroid function. Menstrual irregularities may require oestrogen therapy. The role of GH for the short stature is controversial but has been used in some patients with variable effect. If resistance to GHRH can be demonstrated, there is some logic to this therapy. No treatment has a significant effect on AHO.

Disorders Related to Magnesium Deficiency

Hypomagnesaemia is rare and usually arises as a result of impaired intestinal absorption or increased urinary losses, which can be distinguished by measuring the urinary magnesium–creatinine ratio. Some conditions are associated with hypercalciuria while low urinary calcium excretion is present in others.

Signs and Symptoms

Clinical manifestations are similar to those of hypocalcaemia, partly because hypocalcaemia is often also present, as is hypokalaemia, because of disorders that cause loss of both potassium and magnesium such as diuretic use and diarrhoea. Hypomagnesaemia causes arrhythmia and hypotension and its most common symptoms are neuromuscular irritability, vomiting, anorexia and weakness. Chronic hypomagnesaemia is associated with osteoporosis and urinary calculus.

Magnesium is a ligand for the CaSR and, if plasma magnesium falls, PTH secretion is stimulated in a manner similar to that of hypocalcaemia. Hypomagnesaemia (<0.5 mmol/L, 0.6 mg/dL) inhibits PTH secretion in response to hypocalcaemia. This inhibition is incomplete initially and PTH remains elevated but not as high as would be expected for the degree of hypocalcaemia. As concentrations fall further to 0.2–0.3 mmol/L (0.24–0.36 mg/dL), PTH secretion is inhibited completely and a state of hypoparathyroidism exists [90]. During the initial phase, when PTH is still elevated, resistance to the action of PTH caused either by desensitization or downregulation worsens the hypocalcaemia and the hypocalcaemia is resistant to treatment until magnesium has been restored to normal.

Hypomagnesaemia with Hypocalciuria

Hypomagnesaemia with secondary hypocalciuria (HOMG1) (#602014) was thought to be an X-linked recessive condition since it has a preponderance of males, but it is known now to be autosomal recessive [91, 92] caused by a primary defect in magnesium absorption as a result of mutations in *TRPM6* (*607009), which is involved in active transport of magnesium in the gut (Figure 10.1). Chronic hypomagnesaemia leads to impaired PTH secretion and responsiveness with consequent hypocalcaemia in the early neonatal period. If the patients survive, treatment requires magnesium supplements in large quantity and often twenty times the normal requirements are needed, although this may be difficult because of the secondary diarrhoea that occurs with oral magnesium supplementation. Acquired malabsorption of magnesium can also occur as a result of gastrointestinal pathology such as in Crohn's or Whipple diseases.

Hypomagnesaemia with associated hypocalciuria (HOMG2) (#154020) is a condition of primary renal magnesium wasting caused by misrouting of the γ-subunit of the Na^+/K^+-ATPase on the inner membrane of the renal tubule (Figure 10.1). Mutations of *FXYD2* (*601814), which encodes this protein, cause autosomal dominant hypomagnesaemia with reduced urinary calcium excretion [93].

IRH (HOMG4) (#611718) is an autosomal recessive condition caused by a mutation in *EGF* (*131530) that controls magnesium reabsorption via TRPM6 (Figure 10.1). Isolated hypomagnesaemia is associated with normal plasma and urine calcium but patients have psychomotor retardation and seizures with brisk reflexes, presumably as a result of other effects of EGF [14].

Gitelman syndrome (#263800), sometimes referred to as a benign form of **Bartter syndrome,** is a separate entity although there is clearly some overlap. Mutations in the *thiazide-sensitive sodium chloride transporter (SLC12A3)* (*600968) result in hypokalaemic alkalosis with salt wasting, hypomagnesaemia and hypocalciuria [94]. Patients usually present after the age of five years with episodes of muscle weakness, lethargy, tetany and muscle cramps. Dermatitis may be present and although Gitelman's is described as benign, a prolonged cardiac Q–T interval may give rise to arrhythmias and syncopal attacks. Chondrocalcinosis is a feature that these patients share with others with chronic hypomagnesaemia. Urinary calcium excretion is low. Treatment consists of correcting the biochemical abnormalities, particularly potassium and magnesium deficiencies, with oral supplementation.

Bartter syndrome is characterized by salt wasting, hypokalaemic alkalosis, elevated plasma renin and aldosterone and low blood pressure. Mutations in various genes cause this condition including *SLC12A3* (*600839) for **Bartter type 1** (#601678), *KCNJ1* (*600359) for **Bartter type 2** (#241200), *CLCNKB* (*602023) for **Bartter type 3** (#607364), *BSND* (*606412) for **Barter type 4A** (#602522) and *CLCNKA or B* (*602024 or 3) for **Bartter type 4B** (#613090). All are associated with variable degrees of hypermagnesuria [13].

Other syndromes associated with hypermagnesuria include *KCNA1* (*176260) for **autosomal dominant hypomagnesaemia (episodic ataxia/myokymia syndrome)** (#160120), *KCNJ10* (*602208) for **SeSAME/east syndrome** (#612780), *HNF1β* (*189907) for **renal cysts and diabetes syndrome** (MODY5) (#137920) and *PCBD1* (*127090) for **MODY5-like syndrome** (#264070) [13].

Hypomagnesaemia with Hypercalciuria

CLDN16 (paracellin 1) (*603959), located in the tight junctions of the epithelium of the ascending loop of henle [95], is mutated in **hypermagnesuria with hypercalciuria and nephrocalcinosis (HOMG3)** (#248250). It allows excessive excretion of both magnesium and calcium. Several different homozygous or compound heterozygous mutations have been described and the severity of the condition varies according to genotype. In some cases the problem is self-limiting while in others renal failure may ensue. Hypocalcaemia is occasionally present.

Renal hypomagnesaemia with ocular involvement (HOMG5) (#248190) is autosomal recessive and similar to HOMG3 but it includes ocular abnormalities such as coloboma, myopia and horizontal nystagmus. No mutations are found in the claudin 16 gene but mutations have been identified in *CLDN19* (*610036) [96], located mainly in the collecting ducts of the renal tubule.

Hypomagnesaemia with seizures and mental retardation (HOMG6) (*607803) is autosomal dominant and caused by mutations in *Cyclin M2 (CNNM2)* that has a specific role in magnesium transport in the kidney [13]. It is the commonest form of isolated hypermagnesuria after HOMG1, 3 and 5.

Acquired Hypermagnesuria

Acquired tubulopathies leading to hypermagnesuria may occur in diabetic ketoacidosis, chronic alcoholism or following the chronic use of drugs such as loop diuretics, cyclosporin A, aminoglycoside antibiotics, cisplatin and cetuximab. Treatment is aimed at restoring plasma magnesium concentrations and thereby prevent inhibition of PTH secretion.

Rickets

Rickets is a condition of the growth plate in which the normal columnar arrangement of chondrocytes with progression of chondrocyte development from resting to proliferative to hyperplastic to apoptotic cells is disrupted. Apoptosis normally induces invasion of the growth plate by capillaries that introduce bone-forming cells that convert cartilage to bone. It is induced by phosphate and hypophosphataemia, whatever the cause, is the common factor [56]. Thus rickets can be divided into causes that are secondary to hyperparathyroidism (e.g. vitamin D deficiency) and those that are secondary to raised FGF23 and renal causes of phosphate loss.

Rickets and Other Disorders with Raised PTH Secondary to Abnormal Supply or Metabolism of Vitamin D

Vitamin D deficiency

The definition of vitamin D deficiency is controversial but a consensus suggests that deficiency in children should be considered when 25OHD concentrations are below 30–35 nmol/L (12–14 ng/mL) and that insufficiency is present when above this but below 50 nmol/L (20 ng/mL) [97]. Some adult physicians regard 70–80 nmol/L (28–32 ng/mL) as the lower limit of normal on the grounds that some individuals show increases in PTH below this level, but not everyone with vitamin D deficiency as defined above has symptoms and it is probable that calcium intake plays an important part in determining whether or not problems arise [98]. Calcium deficiency rickets is also described in patients, particularly from West and South Africa and parts of tropical Asia, in whom vitamin D deficiency was relatively unimportant but who had very low calcium intakes [99]. These patients tend to present later than those with vitamin D deficiency and respond better to treatment with calcium supplementation than to vitamin D.

Vitamin D deficiency and nutritional rickets remain significant causes of abnormalities of calcium metabolism. Following the recognition of the importance of vitamin D, rickets was virtually eliminated in western societies with fortification of foods and administration of vitamin D supplements to children but a resurgence of vitamin D deficiency was seen following increased immigration to the UK, particularly from the Caribbean and Indian subcontinent, in the 1950s and 1960s. Various campaigns, such as the Glasgow 'stop rickets' campaign during the 1970s, reduced the incidence of rickets and vitamin D deficiency, but there has been a 'third wave' seen in both the UK and the USA and vitamin D deficiency remains the single most common cause of rickets.

Vitamin D deficiency can also arise as a result of impaired absorption of vitamin D in gastrointestinal disorders such as coeliac disease, especially if the supply of vitamin D from sunlight is restricted.

Disorders Associated with Vitamin D Deficiency

Congenital rickets has occasionally been described in infants whose mothers have undetectable 25OHD. Changes typical of rickets and multiple fractures are seen on X-ray, respiratory function is compromised and respiratory support is usually needed. The condition requires intensive care until vitamin D and calcium supplementation effect bone healing sufficient to allow respiratory function to improve. If patients survive, recovery is usually complete although this may take several weeks.

Post-Neonatal Vitamin D Deficiency

Three conditions have been described in association with vitamin D deficiency in the post-neonatal period. Young infants and adolescents may present with hypocalcaemic convulsions and other symptoms associated principally with hypocalcaemia. Rickets is often not present, particularly in teenagers. The muscle spasm that accompanies the hypocalcaemia can be very painful but is rapidly relieved by intravenous infusion of calcium. This situation arises early in the development of vitamin D deficiency in children who are growing rapidly before rickets has a chance to develop [100]. The biochemical abnormalities are rapidly corrected by high-dose vitamin D with calcium supplements.

Classical '**nutritional rickets**' develops in older infants and toddlers but may also be seen later in childhood. Mild hypocalcaemia, hypophosphataemia and greatly elevated alkaline phosphatase are usually seen and, as the rickets worsen, hypocalcaemia may become symptomatic. Patients present with walking difficulties, caused by muscle weakness, and bowing of the legs. Genu valgum and varum or a combination of the two, giving the so-called 'wind-swept' appearance, are commoner in older children. Swelling of the wrists and knees and a 'rickety rosary' are apparent (Figure 10.13). Developmental delay, particularly of the motor milestones, is often seen. The classical radiological features of rickets become apparent with widening and splaying of the epiphyses with 'moth-eaten' features giving them the so-called champagne glass appearance (Figure 10.14). The bones have an osteopenic appearance and evidence of secondary hyperparathyroidism, including microcysts along the borders of the phalanges and periosteal reaction, can be seen. Fractures may be present [101] in severe cases. Treatment consists of high-dose vitamin D that usually corrects the biochemical abnormalities within a few weeks but rickets takes longer to heal and bowing of the legs and other skeletal deformities may take several months to correct. Orthopaedic procedures should be delayed until it is clear that further remodelling is not going to occur, particularly in very young children.

Dilated cardiomyopathy, a potentially fatal complication of vitamin D deficiency, occurs, albeit rarely, in infants who present with feeding difficulties, respiratory distress and heart failure associated with dilated cardiomyopathy under the age of 6 months [64]. Hypocalcaemia is present and the combination of hypocalcaemia and heart failure should strongly suggest this diagnosis. Urgent treatment with vitamin D is required with supportive therapy for heart failure, which may include extra-corporeal membrane oxygenation. The prognosis is good, unlike that of almost all other kinds of cardiomyopathy in infancy, although, while the biochemical abnormalities are usually correctable within a few days, cardiac function may take months to return to normal. These infants are most frequently born to mothers of South Asian or Afro-Caribbean origin who are themselves vitamin D deficient and the infants are born vitamin D deficient, particularly if breastfed.

In addition to the clearly defined syndromes of vitamin D deficiency, many affected children without overt evidence of rickets or hypocalcaemia complain of vague aches and pains and backache that respond well to treatment.

The biochemical abnormalities of vitamin D deficiency develop in three stages [102] (Figure 10.15) but they are not clearly defined and considerable overlap occurs. In stage 1, hypocalcaemia and hyperphosphataemia are present, bone turnover is increased and alkaline phosphatase is moderately raised. PTH rises secondary to the hypocalcaemia and hypocalcaemic symptoms may be present but rickets is absent. The hypocalcaemia and hyperphosphataemia with raised PTH resembles an acquired PHP-like state and PTH resistance, as demonstrated by impaired cAMP responses to PTH, is present. Because of this, it is not possible to define a cause for hypocalcaemia until vitamin D deficiency has been excluded or corrected. In some parts of the UK, vitamin D deficiency remains the commonest cause of hypocalcaemia outside the neonatal period.

In stage 2, PTH rises further and overcomes the resistance seen in stage 1. Consequently, plasma calcium is only slightly low and hypophosphataemia supervenes in response to the hyperparathyroidism. Alkaline phosphatase increases further as rickets develops.

In stage 3, hypocalcaemia worsens and may again become symptomatic and hypophosphataemia persists. Rickets becomes worse, the radiological appearances become more obvious and alkaline phosphatase rises further.

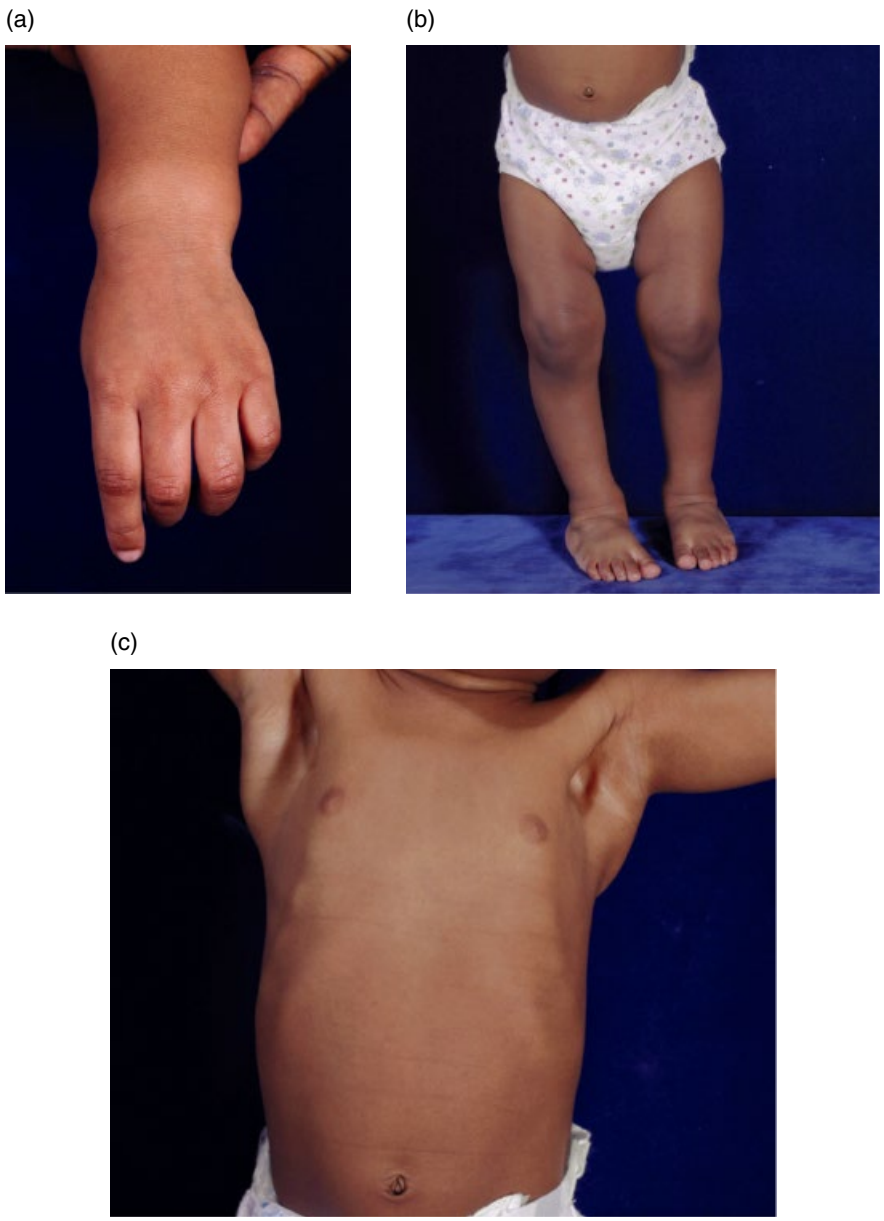


Figure 10.13 Clinical appearances of swelling of the wrists (a), knees (b) and of the 'rickety rosary' (c) in a case of classical vitamin D deficiency rickets. The 'rickety rosary' is seen lateral and parallel to the costal margins. *Source:* Reproduced by kind permission of the patient's family.

In vitamin D deficiency, 25OHD is usually low but a normal concentration does not exclude the diagnosis, especially if vitamin D has been administered or sunlight exposure obtained before presentation. $1\alpha,25(OH)_2D$ (if measured) is also low but may be normal or even elevated if vitamin D treatment has already begun because $1\alpha,25(OH)_2D$ rises to supra-physiological concentrations in response to the high PTH following administration of vitamin D and falls to physiological levels only as the rickets heal [103].

Treatment of vitamin D deficiency is best undertaken with vitamin D and not one of its analogues. A dose of 3000 IU/day in infants up to 6 months of age, 6000 IU/day from 6 months to 12 years and 10,000 IU/day thereafter is usually sufficient to correct the biochemistry and restore vitamin D stores satisfactorily [97, 104]. Acute symptomatic hypocalcaemia may require calcium infusions until symptoms subside and it is advisable to give oral calcium supplements as well as vitamin D. Alfacalcidol should be avoided because it does not

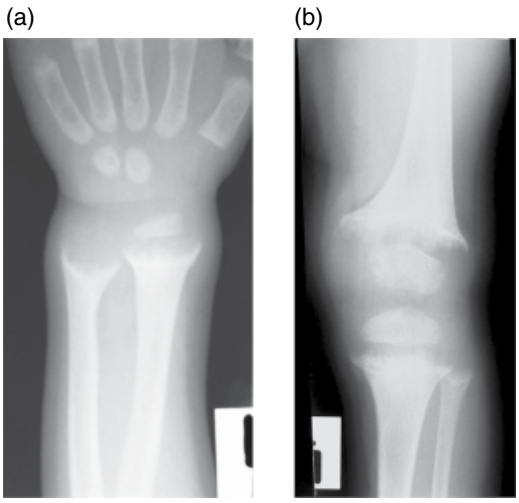


Figure 10.14 X-ray appearances of the wrists (a) and knees (b) in classical vitamin D deficiency rickets. Both show the typical 'champagne glass' appearances of the epiphyses with concave margins and a ragged appearance.

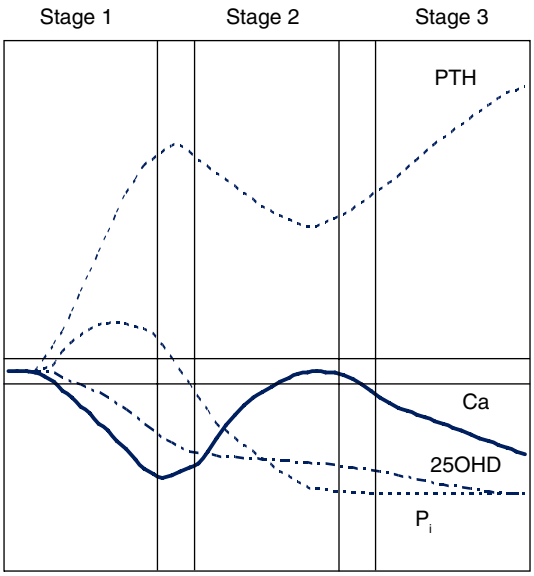


Figure 10.15 Stages in the development of vitamin D deficiency. *Source:* Adapted from Arnaud et al. [102] by permission of De Gruyter.

correct vitamin D deficiency and, if used in 'physiological' doses, may delay healing of rickets because supra-physiological levels are required for adequate healing.

Disorders of Vitamin D Metabolism

The biochemical changes seen in rickets associated with abnormalities of vitamin D metabolism are similar to those seen in vitamin D deficiency, with the exception of the vitamin D metabolites. Chronic liver disease may affect 25-hydroxylation of vitamin D but this is not usually of clinical significance and patients with chronic liver disease are usually given vitamin D supplements. In very low birthweight infants (23–25 weeks' gestation), who often develop a degree of hepatitis, poor 25-hydroxylation may be significant, and these infants sometimes require treatment with calcitriol rather than alfacalcidol to overcome the defect. A rare form of rickets, **selective 25-hydroxy vitamin D_3 deficiency** (VDDR1B) (#600081) caused by mutations in one of the vitamin D 25-hydroxylases, (*CYP2R1*) (*608713), has been described [39]. These patients require treatment with either high-dose vitamin D or calcitriol.

Vitamin D-dependent rickets type 1 (VDRRIA, 1α-hydroxylase deficiency) (#264700) is caused by homozygous or compound heterozygous mutations in *CYP27B1* (*609506) [105, 106] and usually presents during the toddler age with bowed legs and rachitic features. $1\alpha,25(OH)_2D$ concentrations are low or just within the normal range, despite adequate levels of 25OHD and high PTH. Large doses of alfacalcidol (or calcitriol) (150–200 ng/kg/day) may be used in the first instance until the rickets heal, which mimics the supra-physiological concentration of $1\alpha,25(OH)_2D$ that occurs during the initial phase of treatment of vitamin D deficiency. Patients need to be monitored and the dose reduced to prevent hypercalciuria or hypercalcaemia as the bones heal.

Vitamin D-dependent rickets type IIA (VDDR2A), more properly known as **hereditary $1\alpha,25(OH)_2D$-resistant rickets (HVDRR)** (#277440), is caused by mutations in *VDR* (*601769) [106] either within the ligand-binding domain (ligand-binding negative) or the DNA-binding domain (ligand-binding positive). A wide variety of mutations has been demonstrated and, in general, those that are ligand-binding positive have associated alopecia, while those that are ligand-binding negative have normal hair. Patients usually present during early infancy with severe rickets and failure to thrive. Hypocalcaemia, hypophosphataemia and raised alkaline phosphatase are present with radiological signs of rickets. $1\alpha,25(OH)_2D$ is usually raised regardless of whether or not treatment has commenced due to suppressed 24-hydroxylase activity. Treatment with vitamin D analogues further raises concentrations.

Treatment can be very difficult, some patients responding to very large doses of calcitriol, whereas others prove almost completely resistant despite nanomolar concentrations of $1,25(OH)_2D$. The most successful treatment has been with infusions of large doses of calcium that allows mineralization to take place. Once this has been established, it may be possible to maintain satisfactory calcium balance, although this may become more difficult during puberty.

One other form of HVDRR, **vitamin D-dependent rickets with normal vitamin D receptor** (%600785) has been described from South America. Patients present with mainly lower limb deformities but are otherwise

well with normal muscle power and none of the other features normally associated with rickets. Alopecia is not a feature. Plasma calcium is low or low-normal and $1\alpha,25(OH)_2D$ and alkaline phosphatase elevated. No abnormalities have been demonstrated in the VDR and post-translation defects leading to failure of normal protein binding are thought to be the cause.

Renal Tubular Acidosis (RTA)

Renal tubular acidosis can result from defects either in the proximal (type II) or distal (type I) tubule (Table 10.3).

Proximal RTA (pRTA)

The defect in **proximal RTA** results from a low threshold for bicarbonate ions and is usually, though not always, associated with other proximal tubular abnormalities (**Fanconi syndrome**) in which glycosuria, aminoaciduria, proteinuria, phosphaturia and sodium wasting are present. Rickets may result from these defects but is usually associated with hyperphosphaturia. The principal cause involves *SLC4A4* (*603345), which causes AR **pRTA with ocular abnormalities and mental retardation** (#604278). An autosomal dominant form has been less well delineated.

Distal RTA (dRTA)

The defect in **distal RTA** resides in one or some of the mechanisms that promote hydrogen ion excretion through the luminal surface of the tubule. A specific H^+-ATPase pump on the luminal surface contains both A1 and B1 subunits as well as several others. Chloride and bicarbonate are exchanged across the baso-lateral surface through an anion exchanger. Distal RTA is often accompanied by hypercalciuria and a tendency to nephrocalcinosis as well as urinary pH >5.5 and metabolic acidosis usually with hypokalaemia and hypocitraturia. It is important to make the diagnosis and distinguish it from vitamin D-related rickets since treatment with vitamin D or its analogues will worsen the hypercalciuria: the mainstay of treatment should be oral bicarbonate to correct the metabolic acidosis, heal the rickets and diminish hypercalciuria.

Patients present at any age from the neonatal period with failure to thrive and typical rickets. Biochemical investigation shows the presence of metabolic acidosis and, unlike proximal RTA, there is no threshold for acidification of the urine. Vitamin D metabolites are usually normal but hypercalciuria is present and nephrocalcinosis may be seen on renal ultrasound. Alkaline phosphatase is raised in plasma. Hypokalaemia with recurrent episodes of hypokalaemic flaccid paralysis may also be a feature.

Several causes of distal RTA have been described [107] and include autosomal recessive **distal RTA with early-onset recessive nerve deafness** (#267300) caused by mutations in *ATP6V1B1* (*192132), which encodes the B subunit of the apical proton pump mediating distal nephron acid secretion. This is also present in the cochlea and accounts for the progressive deafness.

Autosomal recessive **distal RTA with early- or late-onset deafness (RTADR)** (*192132) is a separate entity caused by abnormalities of *ATP6V0A4* (*605239), which encodes the A subunit of the proton pump. Hearing loss is not usually a feature although it may become so later in life; early-onset has also been reported [108]. The acidosis can become apparent as early as 3 weeks of age.

Both autosomal recessive (#611590) and dominant (#179800) forms of distal RTA have been described in association with mutations in the anion exchanger *SLC4A1* (+109270). This is also present in the red cell membrane where it is known as band 3 protein. A wide variety of mutations in the gene has been identified, as a result of which some patients have predominantly distal RTA while others mainly have elliptocytosis and haemolytic anaemia. The mutations are found mostly in Southeast Asian populations and may confer resistance to malaria.

Patients with *CAII* (*611492) mutations, which causes autosomal recessive **osteopetrosis type 3 (OPTB3)**, have a degree of distal RTA. RTA can also be caused by a variety of acquired conditions including autoimmune disease (e.g. Sjögren's syndrome and systemic lupus erythematosus), drugs (e.g. NSAIDs, heparin and amphotericin B) and obstructive uropathy.

Systemic Conditions Associated with Hypocalcaemia

Tumour lysis syndrome occurs in about 30% of children during the initial phases of treatment of haematological tumours. The release of large quantities of phosphate, potassium and uric acid results in a syndrome characterized biochemically by hyperphosphataemia, hyperuricaemia, hyperkalaemia, uraemia and hypocalcaemia. The hypocalcaemia is largely due to hyperphosphataemia, which occurs secondarily to the acute renal failure of hyperuricaemia. The condition can largely be prevented by a combination of forced alkaline diuresis and the use of the recombinant urate oxidase inhibitor, rasburicase (Fasturtec®), which, although more expensive than allopurinol, has been found to be useful and cost-effective [109].

Chronic kidney disease (CKD) has a serious impact on calcium metabolism. Reduced GFR results in retention of phosphate, plasma concentrations of which begin to rise once GFR falls below 30 mL/min/1.73 m^2. As the kidney is the principal site of 1a-hydroxylase activity, $1\alpha,25(OH)_2D$ falls, particularly when GFR falls below

Table 10.3 Distal renal tubular acidosis and miscellaneous renal tubular disorders associated with hypercalciuria, hypophosphataemia and hypermagnesuria. The metabolic abnormalities, together with the OMIM numbers of the conditions and their genes, modes of inheritance and principal clinical features are shown.

Clinical condition	OMIM	Location	Gene	Gene product	OMIM	Inheritance	Features
Distal renal tubular acidosis							
Autosomal dominant distal renal tubular acidosis	#179800	17q21-q22	*SLC4A1*	Band 3 glycoprotein	+109270	AD	Nephrocalcinosis, nephrolithiasis, rickets
Distal renal tubular acidosis with progressive nerve deafness	#267300	2cen-q13	*ATP6V1B1*	Vacuolar ATPase	*192132	AR	Nephrocalcinosis, rickets, sensorineural deafness
Autosomal recessive distal renal tubular acidosis	#602722	7q33-q34	*ATP6N1B*	Multisubunit H(+)-ATPase pump	*605239	AR	Nephrocalcinosis, nephrolithiasis, rickets
Autosomal recessive distal renal tubular acidosis	#602722	17q21-q22	*SLC4A1*	Band 3 glycoprotein	109270	AR	Nephrocalcinosis, nephrolithiasis, rickets. Elliptocytosis in some patients
Renal tubular acidosis III	267200	?	?	?	?	?AR, ?XL	Rickets, nephrolithiasis, nephrocalcinosis
Other renal tubular disorders causing proximal renal tubular acidosis, hypercalciuria, etc.							
Absorptive hypercalciuria 2	#143870	1q24	*SAC*	Soluble adenylyl cyclase	*605205	AD	Hypercalciuria, recurrent calcium oxalate stones
Absorptive hypercalciuria 1	%607258	4q33-qter	?	?		?	Hypercalciuria, nephrocalcinosis, dysmorphic features
Dent disease 1	#300009	Xp11.22	*CLCN5*	Chloride channel 5	*300008	XLR	Rickets, hypercalciuria, hyperphosphaturia, aminoaciduria, nephrolithiasis, renal failure
X-linked recessive nephrolithiasis	#310468	Xp11.22	*CLCN5*	Chloride channel 5	*300008	XLR	Nephrolithiasis, renal failure
Low molecular weight proteinuria with hypercalciuria and nephrocalcinosis	#308990	Xp11.22	*CLCN5*	Chloride channel 5	*300008	XLR	Low molecular weight proteinuria, hypercalciuria, nephrocalcinosis
X-linked recessive hypophosphataemic rickets*	#300554	Xp11.22	*CLCN5*	Chloride channel 5	*300008	XLR	Hypophosphataemic rickets ± nephrocalcinosis
Dent disease 2	#300555	Xq26.1	*OCRL1*	Phosphatidylinositol 4,5-bisphosphate-5-phosphatase	*300535	XLR	Similar to Dent 1
Lowe oculocerebrorenal syndrome	#309000	Xq26.1	*OCRL1*	Phosphatidylinositol 4,5-bisphosphate-5-phosphatase	*300535	XLR	Vitamin D-resistant rickets, ocular abnormalities, mental retardation
Wilson's disease	#277900	13q14.3-q21.1	*ATP7B*	Copper transporting ATPase Beta polypeptide	*606882	AR	Liver cirrhosis, neurological manifestations, low caeruloplasmin, hypercalciuria, nephrocalcinosis
IMAGE	300290	Chr.X	?	?		XLR	Hypercalciuria, hypercalcaemia, IUGR, adrenal insufficiency, mild dysmorphism, hypogonadotrophic-hypogonadism
Fanconi-Bickel syndrome	#227810	3q26.1-q26.3	*GLUT2*	Glucose transporter 2	*138160	AR	Hypophosphataemic rickets, hepatorenal glycogenosis, proximal renal tubulopathy
Fanconi renotubular syndrome	%134600	15q15.3	?	?		AD	Fanconi syndrome, mild rickets
Cystinosis	#219800	17p13	*CTNS*	Cystinosin	*606272	AR	Hypophosphataemic rickets, metabolic acidosis, photophobia, short stature, hypothyroidism, renal failure

50–60 mL/min/1.73 m^2. Metabolic acidosis, either directly as a result of the CKD or caused by renal tubular disorders that may have led to the CKD, is often a factor. Hypocalcaemia results, which induces secondary hyperparathyroidism. Renal osteodystrophy thus consists of a spectrum of high bone turnover resulting from the hyperparathyroidism and low turnover secondary to the osteomalacia [110]. Additional factors influencing renal osteodystrophy include calcium, phosphorus, vitamin D analogues and aluminium.

The principles of minimizing renal osteodystrophy depend upon preventing hyperphosphataemia, reversing the effects of the reduced 1a-hydroxylase activity and preventing hyperparathyroidism. Oral phosphate-binding agents are used for the former, and sevelamer (Renagel®) is now most commonly used. It is an orally active phosphate-binding agent that is not absorbed and seems to have the advantage over calcium carbonate of not causing adynamic bone while being as effective. Alfacalcidol or calcitriol is used to maintain 1α,25$(OH)_2$D concentrations but treatment must be monitored to prevent hypercalciuria or hypercalcaemia, which might worsen the renal failure. The vitamin D analogue, 19-nor-1α,25$(OH)_2$D2, paricalcitol (Zemplar®), has been used as this has a preferential effect on reducing PTH without increasing renal calcium excretion. The National Kidney Foundation, in their Practical Guidelines for Bone Metabolism and Disease in Children with CKD [111], has also included, as part of their guidelines, the need to ensure that patients with CKD are not vitamin D deficient as this may also cause raised PTH, which may be corrected by vitamin D supplementation alone. Hyperparathyroidism may be effectively controlled by the calcimimetic drug, cinacalcet (Sensipar®, Mimpara®).

Hypercalcaemia

The symptoms of hypercalcaemia in childhood are age dependent. Mild hypercalcaemia may be asymptomatic but as the calcium concentration rises above 3.0 mmol/L, symptoms become more common. It is useful to think about the origin of hypercalcaemia as PTH dependent or PTH independent (normally related to high bone turnover or excessive calcium absorption often via increase in the activity of the vitamin D pathway) and to consider the cause in relation to the age of presentation.

Signs and Symptoms

Infants present with failure to thrive, vomiting, and constipation. Muscle hypotonia, lethargy, anorexia, abdominal pain and constipation may be present in older children. Polyuria and polydipsia result from a concentrating defect in the renal tubule and longstanding hypercalciuria can lead to nephrocalcinosis, kidney stones and renal failure. Occasionally, psychiatric disturbance accompanies hypercalcaemia and reverses when calcium returns to normal. Although not as common as the disorders causing hypocalcaemia, many of those causing hypercalcaemia are also genetic in origin (Table 10.4). Flowchart 10.2 shows the acute management of hypercalcaemia.

Hypercalcaemia in Neonates

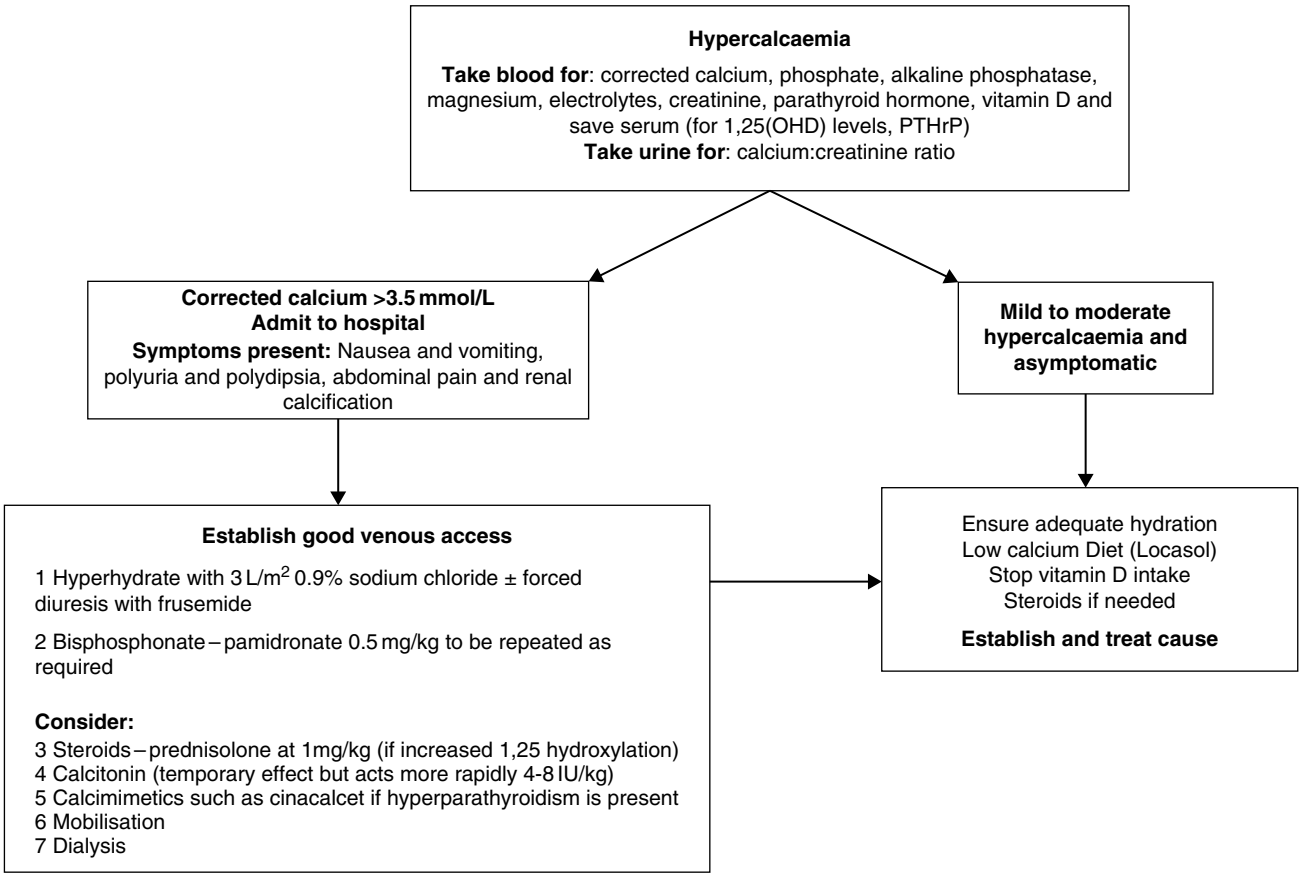
Incorrectly enriched formula feed given to small infants can lead to excess calcium intake and hypercalcaemia. Conversely, decreased phosphate intake in preterm babies can lead to both decreased mineralization and increased free calcium. Low phosphate results in decreased FGF23, which increases conversion of vitamin D to its active form 1,25$(OH)_2$D, which in turn increases intestinal calcium absorption. The PTH in these instances is suppressed.

Subcutaneous fat necrosis occurs in term infants who have suffered a mild degree of birth asphyxia. Firm lumps appear in the subcutaneous tissues and may be multiple. Hypercalcaemia develops within the first few weeks after birth and is accompanied by hypercalciuria and nephrocalcinosis. The skin lesions are invaded by macrophages and the aetiology of the hypercalcaemia is thought to be inappropriate activation of 1alpha-hydroxylase within the macrophages, which results in high concentrations of circulating 1alpha,25$(OH)_2$D [112]. The condition is self-limiting within a few weeks, but steps may need to be taken in the meantime to reduce the plasma calcium level. Calcium and vitamin D restriction, steroids and bisphosphonates may be of value.

Neonatal severe primary hyperparathyroidism (NSPHT) (#239200) is a rare condition usually secondary to a homozygous inactivating mutation in *CaSR* and occurs mostly in consanguineous families [25]. Newborns fail to thrive, feed poorly and suffer constipation and atonia shortly after birth. Gross hypercalcaemia, often in excess of 5.0 mmol/L, and hypophosphataemia are present. PTH is markedly elevated, and hyperparathyroid bone disease develops such that respiratory distress necessitating assisted ventilation may result from poor rib compliance. The bones become thin and develop a 'moth-eaten' appearance because of the severe hyperparathyroidism, which can be identified by the presence of microcysts in the subperiosteal areas. Multiple fractures may occur and be mistaken for rickets. Once the diagnosis has been established, total parathyroidectomy is required to eliminate the hypercalcaemia. Prior to surgery, bisphosphonates may be useful temporarily to restore normocalcaemia. As sometimes happens with primary hyperparathyroidism, a 'hungry bone' condition

Table 10.4 Hypercalcaemic disorders associated with genetic abnormalities. The metabolic abnormalities, together with the OMIM numbers of the conditions and their genes, modes of inheritance and principal clinical features are shown.

Location in calcium cascade	Metabolic abnormality	OMIM	Location	Gene	OMIM	Inheritance	Principal clinical features
Calcium-sensing receptor							
	Neonatal severe hyperparathyroidism – autosomal recessive	#239200	3q21.1	*CaSR*	*601199	AR	Severe hyperparathyroidism, respiratory difficulties
	Neonatal severe hyperparathyroidism – autosomal dominant		3q21.1	*CaSR*	*601199	AD	Severe hyperparathyroidism, respiratory difficulties
	Familial benign hypercalcaemia (familial hypocalciuric hypercalcaemia) type I	#145980	3q21.1	*CaSR*	*601199	AD	Asymptomatic hypercalcaemia
	Familial-isolated hyperparathyroidism						Asymptomatic hypercalcaemia
	Tropical chronic (calcific) pancreatitis	#608189	5q32/3q21.1	*SPINK1/ CASR*	*167790/*601199	AD	
	Familial hypocalciuric hypercalcaemia type II	#145981	19p13.3	*GNA11*	*139313	AD	Asymptomatic hypercalcaemia
	Familial hypocalciuric hypercalcaemia type III (Oklahoma variant)	#600740	19q13	*AP2S1*	*602242	AD	Asymptomatic hypercalcaemia
	Calcium-sensing receptor blocking antibodies						
Disorders of the parathyroid glands/PTH oversecretion							
	Multiple endocrine neoplasia type 1	#131100	11q13.1	*MEN1*	*613733	AD	Primary hyperparathyroidism
	Multiple endocrine neoplasia type 2a	#171400	10q11.21	*RET*	*164761	AD	Primary hyperparathyroidism
	Multiple endocrine neoplasia type 2b	#162300	10q11.21	*RET*	*164761	AD	Primary hyperparathyroidism
	Multiple endocrine neoplasia type 4	#610755	12p13.1	*CDKN1B*	*600778	AD	Primary hyperparathyroidism
	Familial isolated hyperparathyroidism, type 1	#145000	1q31.2	*CDC73*	*607393	AD	Primary hyperparathyroidism
	Familial isolated hyperparathyroidism, type 2 (jaw tumour)	#145001	1q31.2	*CDC73*	*607393	AD	Primary hyperparathyroidism
	Parathyroid carcinomas	#608266	1q31.2	*CDC73*	*607393	AD	Primary hyperparathyroidism
PTH/PTHrP receptor abnormalities							
	Metaphyseal chondrodysplasia, Jansen type	#156400	3p21.31	*PTHR1*	*168468	AD	Hypercalcaemia, short limbs, bone deformities
Abnormal vitamin D metabolism							
	Infantile hypercalcaemia	#143880	20q13.2	*CYP24A1*	*126065	AR	
Miscellaneous causes of hypercalcaemia in childhood							
	Subcutaneous fat necrosis		12q14.1	*CYP27B1*	*609506		Somatic enzyme induction
	Sarcoidosis, other granulomatous diseases		12q14.1	*CYP27B1*	*609506		Somatic enzyme induction
	Humoral hypercalcaemia of malignancy	#163382	12p11.22	*PTHLP*	*168470		Hyperparathyroidism similar to primary hyperparathyroidism



Flowchart 10.2 Acute management of hypercalcaemia in children.

usually develops post-operatively, which requires infusion of large quantities of intravenous calcium to prevent hypocalcaemia until such time as the bones recover.

NSPHT may also develop in infants with a heterozygous mutation of the CaSR gene. It usually occurs in infants who have inherited the gene from an affected father or as a new mutation, the mother being normocalcaemic. The fetus senses the maternal calcium as low and develops a degree of secondary hyperparathyroidism that settles progressively, usually by 6 months of age [113]. Alternatively, the degree of set point abnormality or bone responsiveness to PTH may be responsible. In these cases, conservative management with a combination of vitamin D and cinacalcet may be sufficient until the hypercalcaemia settles spontaneously to a concentration at which it becomes asymptomatic.

Another phenotype similar to FBH is associated with the presence of CaSR-blocking antibodies that lead to secondary hyperparathyroidism [114]. Mutational analysis of the CaSR was negative in all cases, most of whom had other autoimmune conditions such as hypothyroidism or coeliac disease. The principal difference between this and primary hyperparathyroidism was the absence of hypercalciuria, the raised plasma magnesium and normal PTH. The natural history of this condition is not known but there is a likelihood that it may remit spontaneously as the antibodies decline.

Williams–Beuren syndrome (#194050) is usually sporadic but can be autosomal dominant. During infancy, patients have a characteristic phenotype consisting of 'elfin facies' caused by periorbital fullness, a long philtrum, malar hypoplasia and an open-mouthed appearance caused by an arched upper lip and full lower lip. As they get older, features change and become coarsened; some skeletal abnormalities such as radioulnar synostosis may develop [115]. Many patients develop hypercalcaemia during infancy, which rarely lasts beyond the first year. Subsequently, cardiac anomalies are often present, which manifest themselves particularly as subvalvar aortic stenosis or peripheral pulmonary stenosis. Developmental delay is a feature and patients develop a tendency to 'cocktail party' conversation as children and young adults, in which it appears that they are conducting an intelligent conversation which, on reflection, is largely meaningless.

The aetiology of the hypercalcaemia is not clear. Some patients have been thought to have abnormalities of vitamin D metabolism or CT deficiency while hypercalcaemic, whereas others have been found to have no identifiable defect in any of the parameters of calcium metabolism after the hypercalcaemia resolves. Most cases have a microdeletion of chromosome 7q11.23, which encompasses the *elastin* gene (*130160). A number of other genes including *LIM-kinase* (*601329), *RFC2* (*600404), *GTF21RD1* (*604318) and *GTF21* (*601679), which are all located near the elastin gene on the long arm of chromosome 7, have also been implicated. Some of these are expressed in the central nervous system and variations in the extent of the microdeletion presumably account for the variable nature of the condition. Mutations of the *CT receptor* gene (7q21) are not thought to be responsible. How these mutations affect calcium metabolism is uncertain.

Infant patients present with failure to thrive, poor feeding and irritability. Treatment consists of a low calcium diet and a low calcium milk formula (Locasol®) is useful. Where patients live in hard water areas, there may be sufficient calcium in the water to negate the effect of Locasol. If symptoms are severe, a short course of prednisolone, 1 mg/kg/day, is useful and can usually be stopped after a few weeks. Correcting the hypercalcaemia does not seem to have any effect on the progress of the other features of the disease, which may evolve without hypercalcaemia ever having been present. The wide range of clinical features and guidelines for the management of this condition have been produced by the American Academy of Pediatrics (2001) [116] and the Williams Syndrome Guideline Development Group UK (2009) [115] (see also https://www.orphanet/data/patho/Pro/en/WilliamsGuidelines_2010.pdf).

Idiopathic infantile hypercalcaemia (IIH) (143880) was originally described in infants born to mothers who had been ingesting large quantities (up to 4000 IU/day) of vitamin D and the incidence declined with a general reduction in vitamin D supplementation but some cases continued to occur with no evidence of excess vitamin D intake. Familial cases have been described. Some of the features of this condition show similarities to Williams syndrome and can include hypertension, strasbismus, radioulnar synostosis, failure to thrive and poor feeding. The dysmorphic features are usually absent and correction of the hypercalcaemia allows normal development, although the tendency to hypercalcaemia may last beyond the first year. Lack of mutations in the elastin gene allows this condition to be distinguished from Williams syndrome [117].

The aetiology of this condition is uncertain. It has been suggested that an intrinsic hypersensitivity to vitamin D is present and elevated levels of N-terminal PTHrP have been demonstrated during hypercalcaemia in one series. Treatment consists of lowering the plasma calcium with a calcium- and vitamin D-restricted diet, steroids and bisphosphonates if necessary. Cellulose phosphate has been used to limit calcium absorption.

Some cases of what was previously considered to be IIH have now been found to result from mutations in *CYP24A1* (*126065). Autosomal dominant or recessive mutations inhibit 24-hydroxylase activity that converts 25OHD to $24,25(OH)_2D$ and $1,25(OH)_2D$ to $1,24,25(OH)_3D$, which has the net effect of preventing unduly elevated concentrations of $1,25(OH)_2D$. In the absence of the enzyme, $1,25(OH)_2D$ remains elevated and causes hypercalcaemia (#143880). The hypercalcaemia usually settles with vitamin D restriction but hypercalciuria may persist and cause renal stones.

Metaphyseal chondrodysplasia, Jansen type (#156400), is an autosomal dominant condition that presents in the neonatal period with apparent hyperparathyroidism but without detectable PTH or PTHrP. It is characterized by short-limbed short stature caused by abnormal regulation of chondrocyte proliferation and differentiation in the metaphyseal growth plate; craniosynostosis may require neurosurgery. Hypercalcaemia caused by constitutively activating mutations of the PTH/PTHrP receptor usually settles after the neonatal period. Bone turnover is very high. Treatment is difficult but bisphosphonates may reduce bone turnover markers and be of value.

Hypercalcaemia may also be seen in the neonatal period in association with the severe forms of osteopetrosis.

Hypercalcaemia in Older Children

Hypercalcaemia associated with raised PTH

Primary Hyperparathyroidism

Primary hyperparathyroidism is rare in children with ~1/100th of the incidence in adults [118]. It can result from generalized PT gland hyperplasia or from single or multiple adenomas that may be isolated and sporadic or form part of one of the inherited tumour syndromes. The hyperplasia may be associated with heterozygous or homozygous inactivating mutations of *CaSR*.

Familial isolated primary hyperparathyroidism (FIHP) (#145000) may be caused by mutations in either *HRPT2* that causes **hyperparathyroidism–jaw tumour syndrome (HYP-JT)** or in *MEN1* that is responsible for **multiple endocrine neoplasia type 1**. A third locus, mapped to chromosome 2p14-p13.3, has also been described, although the function of the gene is not known.

Familial hyperparathyroidism may also be seen in **MEN2A** and in **MEN4**. Sporadic hyperparathyroidism

has also been seen in connection with mutations in *PTH* or in *PRAD*1. These genes are thought to be oncogenes or tumour suppressor genes. In sporadic cases, a 'single hit' mutation affects a proto-oncogene such as *PRAD1* resulting in preferential growth of a single cell line. In the familial syndromes, a germline 'first hit' mutation affects a tumour suppressor gene and makes the PT (and other) glands susceptible to a 'second hit' [83]. Tumours that arise in familial hyperparathyroidism are usually the result of hyperplasia while those occurring in sporadic cases are adenomas. However, these can be multiple and it is sometimes difficult to distinguish between the two.

Multiple endocrine neoplasia type I (MEN1) (#131100) is characterized by a combination of PT (90% of patients), pancreatic endocrine (40%) and anterior pituitary (30%) tumours. Adrenocortical and carcinoid tumours as well as lipomas, angiofibromas and collagenomas may also occur [119]. The aetiology is probably an inactivating mutation of *MEN1* (131100.0020) located on chromosome 11q13, which normally codes for a tumour suppressor protein MENIN. Nonsense mutations, deletions, insertions, donor splice-site mutations and missense mutations have all been described. Parathyroid tumours are usually the first to present, generally in late adolescence or young adulthood.

Three different variants of **multiple endocrine neoplasia type II (MEN2)** are described. In the most common variant, **MEN2A** (#171400), PT tumours (20%) are associated with MCT and pheochromocytomas [120]. **MEN2B** (#162300) (previously known as MEN3) is not usually associated with PT tumours but has an association with pheochromocytomas, mucosal neurofibromas and intestinal autonomic ganglion dysfunction. In the third variant, **MCT only** (#155240), no tumours other than MCT occur.

All three are linked by mutations in a gene that maps to chromosome 10cen-10q11.2. This contains the *c-ret proto-oncogene (RET)* (*164761). Different mutations have been found in all three variants and identification of them is useful in the diagnosis and management of family members at risk. Mutations in *neurotropic tyrosine kinase receptor type 1 (NTRK1)* (*191315) have been shown to cause isolated MCT [121].

Multiple endocrine neoplasia type IV (MEN4) (#610755) is caused by mutations in *cyclin-dependent kinase inhibitor 1B* (*CDKN1B)* gene (*600778) [122]. Pituitary, PT and other tumours are featured in this condition, although usually only in adulthood. The gene product is thought to be a suppressor of cell proliferation that acts via cyclin-dependent kinases. Allelic loss of chromosome 1p32-pter has been found in a number of cases of isolated sporadic PT adenomas [123]. This region contains a putative tumour suppressor gene.

HYP-JT (#145001) is an autosomal dominant syndrome. Parathyroid adenomas and carcinomas are associated with mandibular and maxillary jaw tumours that are fibro-osseous in nature. Renal tumours are seen on occasions. Different tumours may occur in different members of the same family. Inactivating mutations of the *parafibromin (HRPT2) (CDC73)* gene (*607393), located on chromosome 1q21-q31, are thought to be responsible [124]. The parafibromin protein, together with the protein products of three other genes, *LEO1* (*610507), *PAF1* (*610506) and *CTR9* (*609366), forms a parafibromin complex that associates with RNA polymerase and acts as a tumour suppressor [125].

Parathyroid carcinoma (#608266) can be difficult to distinguish from adenoma histologically, unless metastases are present. It may cause aggressive hyperparathyroidism, which can be difficult to treat, especially once it has metastasized. It may be a feature of HPT-JT syndrome and many patients with sporadic PT carcinoma have deletions in *CDC73* [126]. Hypercalcaemia and hypophosphataemia are associated with raised PTH. Urinary calcium excretion is raised and a partial Fanconi syndrome usually present. Plasma magnesium is often slightly low, in contrast to FHB. Radiological examination may reveal the presence of subperiosteal microcysts and severe hyperparathyroidism can be confused with rickets. Treatment is surgical.

Localization of PT tumours is best undertaken with the aid of radionuclide scanning with ^{99m}Tc-MIBI (methoxyisobutyl isonitrile) or ^{99m}Tc-tetrofosmin (Figure 10.16). These methods are more sensitive than either ultrasonography or magnetic resonance imaging (MRI) and are invaluable in locating persistent tumours, especially after primary surgery has failed to eradicate the problem. They are sometimes combined with thyroid subtraction scintigraphy and, if performed shortly before surgery, can be combined with the use of a handheld gamma camera to pinpoint the tumour at operation. Peroperative measurement of PTH has proved useful in determining whether or not a tumour (or tumours) has been fully resected. Surgery should be undertaken only by surgeons experienced in endocrine tumour resection.

Disorders of the PTH Gene

Sporadic PT tumours may result from mutations within *PTH* itself. *Cyclin D1 (CCND1; PRAD1)* (*168461) is derived from a rearrangement of exon 1 of *PTH*, which is normally not translated, with new non-PTH DNA located on chromosome 11q13. It normally encodes for a 295-amino acid protein, cyclin D1 [127]. Mutations resulting in overexpression of this gene cause PT cell proliferation and hyperparathyroidism. The mutations are somatic and are therefore not inherited.

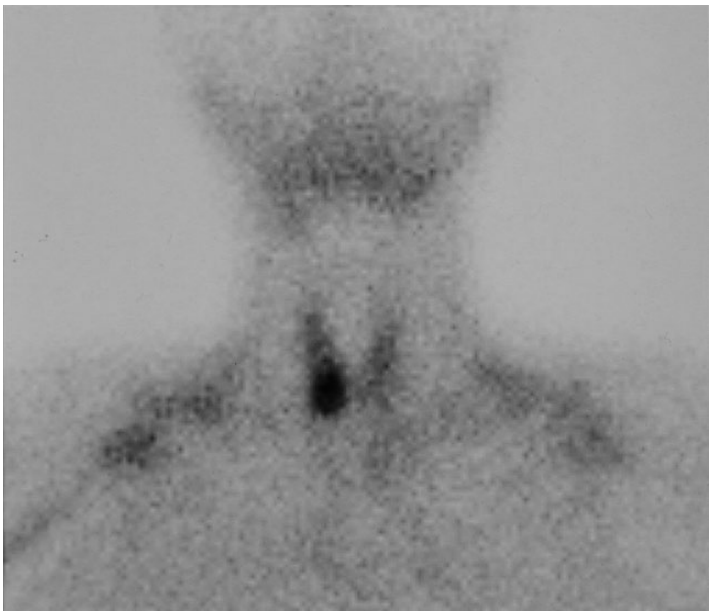


Figure 10.16 SestaMIBI scan taken in a child with a single right lower parathyroid adenoma. She presented with hypercalcaemia. A single adenoma was removed at operation and she remains normocalcaemic. No mutation of either MEN1 or HYP-JT genes has been demonstrated.

A 22-bp deletion within exon 2 of *PTH* has been described [128]. It is possible that silencing of the PTH gene leads to overexpression of *PRAD1/cyclin D1*, thus causing the PT tumours seen in this condition. This may cause confusion when assaying PTH as it may not react in all immunoassays.

Hypercalcaemia Associated with Normal PTH

Familial benign hypercalcaemia (FBH) or **familial hypocalciuric hypercalcaemia (FHH)** (#145980) was described in patients initially thought to have primary hyperparathyroidism but who remained hypercalcaemic despite subtotal parathyroidectomy [129]. Twenty-three asymptomatic family members in three generations were subsequently found to be hypercalcaemic without hypercalciuria. Following the identification of *CaSR*, inactivating mutations were shown to be the cause in most cases (66%). There were a few families in whom mutations in *CaSR* were not demonstrated and these are caused by mutations in either *GNA11* (*139313) [130] (10%) or *AP2S1* (*602242) [131] (20%). These two variants have been designated as **FHH2** (#145981) and **FHH3 (Oklahoma)** (#600740). In all cases it is an autosomal dominant condition and most of the patients are heterozygous. It is often identified incidentally or as a result of investigation of FBH kindreds. In some families, there is a history of parathyroidectomy for presumed hyperparathyroidism. Plasma calcium usually remains elevated throughout life. There is a high degree of penetrance and hypercalcaemia has usually developed before 10 years of age but often much earlier. Most patients remain asymptomatic, although some infants may develop mild symptoms during the first year. In FHH3 PTH may become elevated later in life and that can make it particularly difficult to distinguish from primary hyperparathyroidism. Pancreatitis has been described as a rare complication in FBH but whether this is a true association or whether the hypercalcaemia itself may be the cause is not clear. Some *CaSR* mutations may confer susceptibility to pancreatitis (#608189) in a subgroup of patients associated with mutations in another gene *SPINK1* (*167790).

FBH must be distinguished from primary hyperparathyroidism. Although plasma calcium is elevated, sometimes above 3.0 mmol/L, PTH remains normal unless attempts have been made to reduce the plasma calcium with low calcium diets. The PTH has normal biological activity [132] but, in contrast to hyperparathyroidism, plasma magnesium is usually slightly elevated [133], urinary calcium excretion is inappropriately low for the degree of hypercalcaemia and nephrocalcinosis does not develop. Treatment of FBH is usually unnecessary and when the condition is diagnosed in a child of a kindred known to carry the gene, reassurance is all that is required.

Hypercalcaemia Associated with Suppressed PTH

Increased 1,25(OH)$_2$D Increased endogenous synthesis of 1,25(OH)$_2$D is thought to occur in sarcoidosis although this is rare in childhood. 30–50% of children with sarcoidosis develop hypercalcaemia, which may be precipitated by sunlight. Others have hypercalciuria without hypercalcaemia. Other granulomatous diseases, including tuberculosis and cat-scratch disease, may also cause hypercalcaemia via a similar mechanism. The hypercalcaemia usually resolves with treatment of the underlying condition.

In very large doses, vitamin D may cause hypercalcaemia, mainly because of the high concentrations of 25OHD that result from the uncontrolled metabolism of vitamin D by 25-hydroxylase. Although 25OHD has limited activity, high concentrations (usually over 250–300 nmol/L) cause bone resorption. A more common cause of hypercalcaemia as a result of vitamin D excess is seen in patients treated with overdosage of either alfacalcidol or calcitriol. The symptoms are those typical of hypercalcaemia from other causes; prolonged hypercalcaemia may cause ectopic calcification, nephrocalcinosis and impaired renal function. Hypercalcaemia following excess vitamin D is usually more prolonged than that caused by the vitamin D metabolites because vitamin D itself is stored in fat, whereas the metabolites have a much shorter half-life. Treatment is directed towards restricting the source of excess vitamin D. If acute symptoms are present, steroids or bisphosphonates may be of value.

Increased Bone Turnover **Immobilization hypercalcaemia** occurs in a small proportion of patients with quadriplegia or other neurological insults [134]. It is more common in adolescents whose bone turnover is naturally more rapid than in adults. The symptoms of lethargy, mood changes, nausea, vomiting and anorexia are nonspecific but may be overlooked in the context of the other problems. They usually arise within a few days or weeks of the original insult when bone turnover is very rapid. Hypercalcaemia and hypercalciuria are present, and nephrocalcinosis can result. Bone biopsy shows loss of trabecular volume, increased osteoclast and decreased osteoblast activity with an overall increase in bone turnover. The aetiology is probably multifactorial and includes lack of mechanical stress, poor vascularity and metabolic changes in bone and denervation [134]. If remobilization is not possible and conventional treatment with intravenous fluids and loop diuretics (which may increase urinary calcium excretion) are ineffective in controlling the hypercalcaemia, infusion of pamidronate 0.5 mg/kg daily for 2–3 days usually works. This effect may last for several weeks but may need to be repeated. CT has been used but the effect is not as rapid and it has to be given in divided daily doses for longer. The hypercalcaemia is self-limiting as bone turnover slows. Other conditions with increased turnover that increases calcium release include thyrotoxicosis [135] and vitamin A toxicity [136].

Hypercalcaemia of Malignancy

Hypercalcaemia is a rare complication of malignancy in childhood and has been reported to occur in about 0.4% of cases. It may be a presenting feature of leukaemia but also occurs in Hodgkin's disease, non-Hodgkin's lymphoma and a variety of solid tumours, such as rhabdomyosarcoma, hepatoblastoma, neuroblastoma and angiosarcoma [137]. Various causes are known: in acute lymphoblastic leukaemia, localized osteoclastic bone resorption in the bone marrow space surrounding malignant cells leads to hypercalcaemia [138] and, as with other conditions complicated by hypercalcaemia, the symptoms may be overlooked. **Humoral hypercalcaemia of malignancy** is related to excess secretion of PTHrP and PTH is low. Bone turnover is increased and if the hypercalcaemia does not remit on treatment of the underlying malignancy, it usually responds well to bisphosphonate. Some tumours, particularly non-Hodgkin's lymphoma, secrete $1,25(OH)_2D$ that causes hypercalcaemia [139] and ectopic PTH secretion is known as a rare cause in adults but has not been described in children.

Hypercalcaemia can also occur in both acute and chronic renal failure. In the former this is usually during the recovery phase while in the latter it may be the result of secondary (or tertiary) hyperparathyroidism.

Hypercalcaemia may follow bone marrow transplantation in some forms of **osteopetrosis**. This is usually an indication of a successful transplant and that osteoclast function is being restored. It may require temporary treatment with denosumab or bisphosphonates.

Management of Hypercalcaemia

Management of hypercalcaemia consists of correcting any dehydration, decreasing calcium and vitamin D intake and trying to correct underlying disorders.

Decrease Gut Absorption of Calcium

Steroid treatment with hydrocortisone or prednisolone decreases conversion of 25OHD to $1,25(OH)_2D$, thus decreasing gut absorption of calcium. Oral phosphate supplementation lowers serum calcium by binding calcium and increasing FGF23, which in turn inhibits $1,25(OH)_2D$ production. In the medium and long term, a low calcium formula preparation is available.

Increase Renal Excretion

In the acutely unwell child increasing urinary calcium excretion and correcting the dehydration that often accompanies hypercalcaemia is the first line of management. Hyperhydration using volumes of $3\,L/m^2/24\,h$ of saline infusion and using diuretics such as furosemide to inhibit proximal and loop of Henle sodium reabsorption in the kidneys will bring the calcium down. Diuretics should be used only in the acute situation, since furosemide increases the risk of nephrocalcinosis. Hyperhydration is usually enough to reduce calcium levels unless a particularly aggressive pathology is present.

Decrease Bone Turnover

Calcium release from the skeleton contributes to circulating calcium concentrations and acute use of bisphosphonates can be very effective in managing moderate to severe hypercalcaemia. Pamidronate intravenously (0.5–1.0 mg/kg over 4–6 hours) can be used either as a one-off dose or repeated if needed. The effect is seen over the next 12–24 hours and most pronounced on day 3. It should be avoided if eGFR <30 mL/min/1.73 m^2. Denosumab (Prolia®, Xgeva®), a RANKL inhibitor, can be used as an alternative if renal failure (eGFR < 35 mL/min/1.73 m^2) is present. CT (5–10 units/kg every 6–12 hours) has multiple effects to reduce serum calcium including reducing bone turnover and can also be considered in resistant cases. Rapid initial responses later diminish.

Reduction of PTH

If hyperhydration is inadequate in hyperparathyroidism, steps to reduce PTH secretion can be taken. The calcimimetic cinacalcet (Mimpara®) (starting dose 0.8 mg/kg/day) [140] reduces PTH secretion and has been used mainly in the management of secondary hyperparathyroidism but it can be used as an adjunct in the management of severe hypercalcaemia associated with hyperparathyroidism such as in NSHPHT.

Dialysis and Parathyroidectomy

In cases of PT adenomas or congenital homozygous *CaSR* mutations, treatment consists of surgical removal of the tumours or PT glands, which should be undertaken only by those experienced in the procedure [118]. It may be necessary to control hypercalcaemia with forced diuresis and frusemide before surgery and bisphosphonates (e.g. pamidronate given in a dose of 0.5 mg/kg daily for 2–3 days) may also be required to normalize plasma calcium. Plasma calcium usually declines post-operatively within a few hours and the patient may become hypocalcaemic and remain so for some time if hyperparathyroidism has been longstanding. In this case, a 'hungry bone' syndrome develops that requires infusion of calcium in large quantities. Dialysis is rarely needed but may be necessary as a temporary measure where the initial steps have not been successful and clinically the patient is very unwell.

Tertiary Hyperparathyroidism

This occasionally occurs in children after chronic hyperstimulation of the PT glands particularly in CKD. It may also rarely result from chronic vitamin D deficiency and can be a complication of X-linked dominant hypophosphataemic rickets, even if appropriately treated. Hyperparathyroidism is also a feature of familial hyperphosphataemic tumoural calcinosis type 3 caused by mutations in *Klotho*. PTH is usually very elevated and the hyperplastic glands become susceptible to developing autonomous nodules. It is not clear whether or not this adenomatous formation is polyclonal or monoclonal but the latter may be present in a majority of cases. Treatment consists of parathyroidectomy.

Hypophosphatasia

This rare condition is usually autosomal recessive and caused by mutations in *tissue non-specific alkaline phosphatase (TNSALP)* (171760). Six different conditions are described depending on the age of presentation and severity [141, 142]. Hypercalcaemia and hyperphosphataemia occur in the more severe forms.

In **perinatal hypophosphatasia**, infants are born with undermineralized bones with rachitic changes, have a high-pitched cry and unexplained fevers and seizures and usually die shortly after birth. This is usually the homozygous form of the condition. A benign form of perinatal hypophosphatasia is also described in which the initial severe symptoms gradually settle. This is usually associated with heterozygous mutations in *TNSALP*. In **infantile hypophosphatasia** (#241500), hypercalcaemia and its attendant symptoms and bone abnormalities develop during the first 6 months. Initial development may be normal until the onset of symptoms and, if they survive, there tends to be gradual improvement with time. Raised intracranial pressure may result from cranial synostosis. **Childhood hypophosphatasia** (#241510) presents later and is very variable in its manifestations. It is often accompanied by premature tooth loss, which is distinguished from normal loss of deciduous teeth by minimal tooth root resorption. Short stature is often a feature and charactistic appearances are seen on X-ray. There are 'tongues' of demineralized bone seen at the metaphyses. However, the characteristic widening of the growth plate, as seen in rickets, is absent. A dolichocephalic shape to the skull may be caused by craniosynostosis. The condition tends to improve at adolescence, although osteomalacia may reappear later in life. **Adult hypophosphatasia** (#146300) is relatively mild but there may be a history of tooth loss and 'rickets' during early life. Diagnosis is made by a combination of a low alkaline phosphatase and characteristic radiological and clinical features. Raised levels of phosphoethanolamine and pyridoxal phosphate are found as both are substrates for alkaline phosphatase. In **odontohypophosphatasia,** premature tooth loss is the only feature and **pseudohypophosphatasia** is characterized by infants who appear to have the clinical features of hypophosphatasia but with normal alkaline phosphatase. This is thought to be caused by an abnormally inactive enzyme.

Treatment is mainly symptomatic: raised intracranial pressure may require neurosurgical intervention; orthopaedic surgery, physiotherapy and dental care may be needed. Infusions of plasma containing high concentrations

of alkaline phosphatase have been tried without success. Bisphosphonates are contraindicated because they are pyrophosphate analogues that inhibit bone mineralization. Subcutaneous injection of asfotase alfa (Strensiq®), a bone-targeted form of alkaline phosphatase, has shown promising results [143] and been approved for use in both the USA and Europe for perinatal, infantile and juvenile hypophosphatasia.

Disorders of Bone Metabolism

The metabolic disorders that directly affect bone can roughly be divided between those that affect bone matrix and those that result from poor bone mineralization. Most conditions in both groups result in changes in bone density but those that demonstrate uniform changes in bone density are referred to as the osteoporoses while those in which mineralization defects are paramount are known as the osteomalacias. In children, osteomalacia is most obviously apparent in the growth plates and gives rise to rickets, which cannot occur in adults once the growth plates have fused. There are several conditions in which changes in bone density occur in specific areas leaving the remainder of the bone intact.

Investigation of Bone Metabolic Disorders

Clinical Assessment

The diagnosis of osteoporosis in children is primarily clinical, supported as necessary by radiological, bone density and genetic data. When a child presents with multiple fractures, particularly if they originate from mild trauma, a careful history, including family history, is taken. Biochemical investigation includes measurement of a bone profile with PTH and 25OHD as a measure of vitamin D status. All these parameters are usually normal, although urinary excretion of calcium may be elevated, particularly in **idiopathic juvenile osteoporosis (IJO)** (259750) and those forms of OI where growth is poor. Blood may be taken for DNA analysis [78, 144]. Conditions associated with high bone density may present with fractures; X-rays will usually give a clue to the diagnosis, which can then be confirmed with biochemical and genetic tests.

Bone Turnover Markers

Two types of marker are used, those that reflect bone formation and those that result from bone resorption [145]. Bone formation markers derived from osteoblast activity are present as type 1 collagen propeptide (P1CP), bone-specific alkaline phosphatase (bALP) or osteocalcin (bone Gla-protein). P1CP is derived from cleavage of the propeptide sequence of type 1 collagen as it is laid down to form bone matrix. Measurement of bALP relies on its greater heat lability as compared with other forms of ALP, and osteocalcin is derived solely from bone and escapes into the circulation at the time of matrix formation.

Bone resorption markers are derived from the products of matrix removal. Most common are urinary N- or C-terminal peptides of collagen (NTX-1, CTX-1) that are related to creatinine to allow for variations in urine concentration. Tartrate-resistant acid phosphatase (TRAP) is specifically secreted by osteoclasts and may give a measure of osteoclast number. It is particularly useful in determining the effectiveness of bone marrow transplantation in osteopetrosis. Urinary pyridinoline, deoxypyridinoline and hydroxyproline are rarely used.

Markers of bone turnover may be used for a diagnosis or for monitoring treatment. Thus, OI is usually accompanied by high and IJO by low bone turnover. Bisphosphonates reduce the rate of bone turnover.

Bone Density

Bone density rises gradually during childhood, increases sharply during adolescence and continues to rise more slowly in the late teenage years before reaching a peak in the early twenties. Routine X-rays give a poor indication of osteoporosis because 30–50% of bone mineral has to be lost before it becomes radiologically apparent. They are useful in demonstrating fractures, fracture healing and vertebral morphology and will often give an indication of the presence of increased bone density.

Several methods of bone density measurements are available but most widely used is dual energy X-ray absorptiometry (DEXA) [146, 147] that has a particular advantage in that it delivers a low radiation dose so repeat measurements can be undertaken with safety. In principle, it uses X-rays of two different energies to determine the amount of bone mineral by differential absorption but while DEXA is potentially useful in children because of the rapidly changing density during the phases of growth in adolescence, there are considerable pitfalls in interpretation. Several methods have been developed to take into account the fact that DEXA gives a two-dimensional measurement and to allow for variations in height, age, gender and pubertal status but the best method has yet to be determined [147, 148]. It is particularly important that the use of the T score, which relates bone density to the peak bone density achieved in adults, is avoided in children. Bone densities are usually reported as an age-related Z score, although the bone mineral apparent density (BMAD), which adjusts for size, may be more appropriate in small children, and serial DEXA measurements on the same child are often useful. Only in recent years has the quality of DEXA allowed its use in children for vertebral fracture assessment (VFA). In some hospitals this has replaced lateral X-rays since the radiation dose is substantially lower [146].

Other methods used to assess density include peripheral quantitative computerized tomography (pQCT) and ultrasound. High-resolution pQCT (HRpQCT) has been used to provide detailed bone morphometry as it is able to resolve <100 μm but it has not been fully evaluated and remains a research tool. QCT of the spine gives more precise measurements than DEXA, particularly as it provides a three-dimensional measurement, but it delivers too high a radiation dose for routine use. Whichever method is used, bone density does not necessarily relate to bone strength, particularly in children, in whom the definitions of osteopenia and osteoporosis, as defined in adults, do not necessarily apply.

Bone Biopsy

Biopsy of bone can be useful where there is doubt about a diagnosis [149]. It is usually performed by taking a full thickness biopsy through the iliac crest that includes both inner and outer cortices as well as the intervening trabecular bone. Most value is gained if the patient has had tetracycline labelling during the three weeks before biopsy because tetracycline is laid down on mineralization fronts and, when viewed under UV light, can give quantitative information on bone activity. Biopsies can also be viewed in polarized light to get information about the lamellar pattern and may provide a definitive diagnosis (e.g. in OI type VI).

Disorders of Bone Matrix Accompanied by Low Bone Density

Osteoporosis

Osteoporosis should never be diagnosed in children solely on densitometry but on the presence of vertebral compression fractures (in the absence of high energy trauma or local disease) or BMD Z score ≤−2 with two or more long bone fractures in association with minimal trauma. In clinical practice osteoporosis, especially in chronic disease, is often overlooked and a delayed presentation such as vertebral compression fractures can easily be missed unless specifically screened for as children are not always aware of back pain.

The causes of osteoporosis can be broadly divided into childhood-onset primary osteoporosis, where there is impairment to intrinsic bone accrual, and secondary osteoporosis, where increased fragility is a result of external factors, but there is a large overlap between the two entities.

Signs and Symptoms

42% of boys and 27% of girls will have sustained a fracture by the age of 16 years [150] so when a child presents with recurrent fractures, it is not always easy to establish if these are pathological. History and examination can give clues to the aetiology. History should include a detailed family history of skeletal disorders, the number of fractures with their mechanism and location, any history of joint hypermobility, chronic disease and dental abnormalities. Dentinogenesis imperfecta, appearing as discoloured, translucent teeth and blue sclerae are suggestive of OI. Low bone mass index, fine lanugo hair and delayed puberty suggests an eating disorder. Long bone bowing is suggestive of OI type III or VI, hypophosphataemia or rickets. Absence of clavicles suggest cleidocranial dysostosis and dysmorphic features are suggestive of Hajdu-Cheney syndrome. Blindness in **osteoporosis–pseudoglioma syndrome** (OPPG) and contracture in **Bruck syndrome** clearly point to a diagnosis. Clinical examination should include assessment of auxology, pubertal status, bony deformity, scoliosis, dentition, scleral hue, head shape, locomotor functional ability and pain.

Childhood-Onset Primary Osteoporosis

The most common and important cause of monogenic osteoporosis is OI, which has an incidence of ~6–7/100,000. This is a group of heterogeneous conditions resulting from intrinsic defects in bone matrix. The most common are caused by quantitative or qualitative abnormalities in bone collagen. The original classification [151] divided OI into four types based on clinical differences but progress in understanding the underlying genetic mutations has added several more OI types resulting in confusion. In 2014 a reclassification with five categories was proposed based on clinical and radiological signs [152].

Inheritance of OI is predominantly autosomal dominant but, within the moderate to severe types of OI, there are mutations in genes involved in post-translational modification and chaperoning of collagen type I that are inherited as autosomal recessive diseases.

Types of OI by Clinical Classification (Table 10.5)

Osteogenesis imperfecta type I (OI1) (#166200) is the mildest form of autosomal dominant OI and results from a quantitative defect in either *COL1A1* (*120150) or *COL1A2* (*120160). It is sometimes associated with **dentinogenesis imperfecta** (DI), which distinguishes type Ia from Ib. Blue sclerae are frequently present. These can be difficult to assess but a standardized tool has been developed to overcome the subjective nature of assessment [153]. Fractures occur with minimal trauma but do not usually result in bone deformity if treated adequately. Fractures are uncommon in infancy but occur throughout childhood, usually with minimal trauma. The frequency tends to diminish during childhood and increases again during adolescence before reducing during adulthood although it increases again in women after the menopause and later in men [154]. Hearing difficulties, usually conductive, may develop during adolescence and progress to profound deafness

Table 10.5 Genetic classification of osteogenesis imperfecta. The metabolic abnormalities, together with the OMIM numbers of the conditions and their genes, modes of inheritance and principal clinical features are shown.

Condition	OMIM	Gene location	Gene	Gene product	OMIM gene number	Inheritance	Severity and clinical features
Osteogenesis imperfecta type 1				Type 1A1 collagen			
Osteogenesis imperfecta type Ia – without dentinogenesis imperfecta	#166200	7q22.1; 17q21.31-q22	*COL1A1*; *COL1A2*	Type 1A1 collagen	*120150; *120160	AD	Mild to moderate. May have dentinogenesis imperfecta (type IA) or not (type IB)
Osteogenesis imperfecta type Ib – with dentinogenesis imperfecta	#166200	7q22.1; 17q21.31-q22	*COL1A1*; *COL1A2*	Type 1A1 collagen	*120150; *120160	AD	
Osteogenesis imperfecta type II	#166210	7q22.1; 17q21.31-q22	*COL1A1*; *COL1A2*	Type 1A1 and 1A2 collagen	*120150; *120160	AD	Severe, usually lethal
Osteogenesis imperfecta type III	#259420	7q22.1; 17q21.31-q22	*COL1A1*; *COL1A2*	Type 1A1 and 1A2 collagen	*120150; *120160	AD	Severe, multiple fractures and deformities at birth
Osteogenesis imperfecta type IV	#166220	7q22.1; 17q21.31-q22	*COL1A1*; *COL1A2*	Type 1A1 and 1A2 collagen	*120150; *120160	AD	Mild to moderate, often associated with short stature
Osteogenesis imperfecta type V	#610967	11pter-p15.4	*IFITM5*	Interferon-induced transmembrane protein 5	*614757	AD	Moderate, radio-ulnar membrane with impaired supination, exuberant callus formation
Osteogenesis imperfecta type VI	#613982	17p13.3	*SERPINF1*	Pigment epithelium-derived factor (PEDF)	*172860	AR	Moderate, bone deforming, characteristic 'fish-scale' appearance on bone biopsy
Osteogenesis imperfecta type VII	#610682	3p22, 3p24.1-p22	*CRTAP*	Cartilage-associated protein	*605497	AR	Variable, usually mild to moderate. rhizomelia, coxa vara
Osteogenesis imperfecta type VIII	#610915	1p34	*LEPRE1*	Prolyl 3-hydroxylase 1 (P3H1)	*610339	AR	Severe growth deficiency, extreme skeletal undermineralisation, bulbous metaphyses
Osteogenesis imperfecta type IX	#259440	15q22.31	*PPIB*	Cyclophilin B (CyPB)	*123841	AR	Moderate to severe. Can cause perinatal lethality
Osteogenesis imperfecta type X	#613848	11q13.5	*SERPINH2*	Heat shock protein 47 (HSP47)	*600943	AR	Severe form with abnormal facies and multiple deformities and fractures. Dentinogenesis imperfecta (DI) and renal stones
Osteogenesis imperfecta type XI	#610968	17q21.2	*FKBP10*	Peptidyl-prolyl cis-trans isomerase	*607063	AR	Severe progressive deforming. Can segregate with epidermolysis bullosa simplex
Osteogenesis imperfecta type XII	#613849	12q13.13	*SP7*	Osterix	*606633	AR	Mild form with recurrent fractures, white sclera and no DI
Osteogenesis imperfecta type XIII	#614856	8p21.3	*BMP1*	Bone morphogenetic protein 1	*112264	AR	Associated with normal or high bone mineral density. Moderate to severe disease with recurrent multiple fractures
Osteogenesis imperfecta type XIV	#615066	9q31.2	*TMEM38B*	Trimeric intracellular cation channel B (TRIC-B)	*611236	AR	Variable degree of severity of multiple fractures and osteopenia with normal teeth, sclerae and hearing
Osteogenesis imperfecta type XV	#615220	12q13.12	*WNT1*	Wingless-type MMTV integration site family, member 1	*164820	AR	Moderate to severe disease with bone deformity, developmental delay, short stature and normal teeth and hearing
Osteogenesis imperfecta type XVI	#616229	11q11	*CREB3L1*	Old astrocyte specifically induced substance (OASIS)	*616215	AR	Severely affected and perinatal lethal

later in life. Patients may bruise easily and have hypermobile joints. Excessive sweating is a characteristic feature. Growth is usually normal. Radiological evidence of Wormian bones may be present at birth [155].

Osteogenesis imperfecta type II (OI2) is the most severe form of OI and usually fatal. It can be due to autosomal recessive or dominant mutations in genes encoding *COL1A1* and *COL1A2* and those involved in folding of the collagen molecule such as *LEPRE1* (*610339), *CRTAP* (*605497) and *PP1B* (*123841). If the infants are born alive, they have multiple fractures and very poorly mineralized bones. Respiratory problems usually supervene rapidly because of the poor rib compliance.

Osteogenesis imperfecta type III (OI3) (#259420) is the most severe form of OI compatible with life. Affected infants suffer multiple fractures *in utero* and are born small for gestational age. They continue to have multiple fractures with very mild trauma. It is commonly caused by a qualitative defect in *COL1A1* or *COL1A2*. The sclerae are blue at birth but may whiten with age. The bones become progressively deformed and multiple orthopaedic procedures are frequently needed. Growth is often very poor and raised urinary calcium excretion may cause nephrocalcinosis. A characteristic radiological feature is the development of 'popcorn bones' [156] that are not present at birth but increase in number and severity during childhood and disappear as growth ceases. They occur within the epiphyses and metaphyses always in close proximity to the growth plate and appear to be detached fragments of growth plate probably in response to trauma. They mostly occur in the lower extremities, particularly the lower femur and upper tibia, and less frequently in the upper limbs. Bisphosphonate therapy has reduced their incidence.

Osteogenesis imperfecta type IV (OI4) (#166220) is an intermediate form of OI with variable degrees of bone deformity. The sclerae are typically white but may have a bluish tinge. Fracture rates are variable and may not be troublesome but growth is often impaired and it may be this rather than fractures that more concern patients. It is caused by qualitative defects in either *COL1A1* or *COL1A2*.

Osteogenesis imperfecta type V (OI5) (#610967) is moderately deforming. It differs from other forms of OI in that no mutations in either *COL1A1* or *COL1A2* have been demonstrated. One particular mutation in *IFITM5* (*614757), which is a specific osteoblast protein, has been shown to cause this condition. Patients have limited supination of the radius and ulna caused by an interosseous membrane between the bones with associated radial head dislocation, which progresses with age. They have exuberant callus formation both at the site of fractures and elsewhere and this can present as hard lumps, which can be confused with osteosarcoma. Callus progresses initially but may eventually disappear [157]. A third feature is the presence of a radio opaque metaphyseal band adjacent to the growth plate. Rickets-like appearances at the metaphyses are described in the first year of life [158].

Autosomal Recessive Osteogenesis Imperfecta

The autosomal recessive forms of OI can be grouped as mutations in genes that are important in:

1) Chain alignment and helical folding of the 1 α collagen chain. 3-Prolyl hydroxylation of a specific residue by an enzyme complex is important for this and mutations in *CRTAP* (*605497), *LEPRE1* (*P3H1* *610339) and *PPIB* (*123841) disrupt this causing **OI type VII** (OI7; #610682), **OI type VIII** (OI8; #610915) and **OI type IX** (OI9; #259440), respectively. These are all phenotypically severe.
2) Quality control of the collagen triple helix. Mutations in *SERPINH1* (*600943) and *FKBP10* (*607063) are important in this function and cause **OI type X** (OI10; #613848) and **OI type XI** (OI11; #610968), respectively. These also have a severe phenotype.
3) Post-translational processing of procollagen type I chains, such as cross linking in bone via hydroxylation of lysine residues in triple-helical telopeptides. Mutations in *FKBP10* (*607063) also cause **Bruck syndrome type I** (BRKS1) (#259450) and *PLOD2* (*601865) mutations cause **Bruck syndrome type II** (BRKS2) (#609220), in which bone fragility and scoliosis are associated with contractures and pterygia. *BMP1* (*112264) is a metalloprotease that cleaves the C-terminus of type I procollagen and mutations cause **OI type XIII** (OI13) (#614856), which is associated with a high bone mass.

Other genetic causes, associated with phenotypes that are similar to OI type III or IV, include mutations in *SERPINF1* (*172860), encoding the pigment endothelium-derived factor (PEDF), which cause **OI type VI** (OI6) (#613982) characterized by a mineralization defect on bone biopsy; *Osterix* (SP7) (*606633), which is an osteoblast-specific transcription factor that, when mutated, causes **OI type XII** (OI12) (#613849); *TMEM38B* (*611236), which encodes an intracellular monovalent cation channel that maintains intracellular calcium release causing **OI Type XIV** (OI14) (#615066) when mutated; and *CREB3L1* (*616215), which encodes a transcription factor which binds to the promoter region of *COL1A1* and causes **OI Type XVI** (OI16) when mutated (#616229).

Other Monogenic Osteoporotic Conditions

Mutations in WNT/β catenin pathway signalling

The WNT/β catenin pathway is important in osteoblast and bone formation. *LRP5* (*603506) is present on the cell

surfaces of osteoblast precursors and forms a receptor complex with frizzled-related protein for which Wnt (the homologue of the drosophila 'wingless' gene) is the ligand. This results in transformation of preosteoblasts to osteoblasts (Figure 10.6). Activating and inactivating mutations in *LRP5* are recognized to cause high and low bone mass.

Heterozygous carriers of *LRP5* mutations have low bone density and 5% of cases of IJO (259750) have been associated with them. They have a prepubertal onset with vertebral compression and metaphyseal fractures, which resolve by adulthood. Other than *LRP5* mutations, other causes of IJO are not known but several other genes confer susceptibility to osteoporosis.

OPPG (#259770) shares many of the clinical features of mild to moderate deforming OI. The principal defect lies with bone formation within the osteoblast. It is an autosomal recessive condition caused by mutations in *LRP5* (*603506). LRP5 is present in many other tissues, notably retinal vessels, and OPPG is associated with severely reduced vision caused by the presence of pseudoglioma of the retina that results from disordered retinal development. The term pseudoglioma, or **familial exudative vitreoretinopathy** (FEVR), of which five types are described, is a non-specific expression used to describe any lesion that resembles retinoblastoma. In OPPG this form is known as **FEVR4** (#601813). When bilateral, it must be distinguished from other causes of FEVR such as **Norrie disease** (#310600), which is X-linked and not associated with low bone density. OPPG patients present with early-onset low trauma fractures that are often deforming but of variable severity. Vision is impaired and most patients are registered blind although some patients can attend mainstream school. Treatment is undertaken along similar lines to that of OI and responds well to bisphosphonates.

WNT1 Recessive *WNT1* (*164820) mutations have been reported to be an autosomal recessive cause of **OI type XV** (OI15) (#615220), which includes bone fragility and mental retardation, while dominant heterozygous mutations appear to cause early-onset osteoporosis.

PLS3 *PLS3* (*300131) encodes plastin-3 and mutations cause a childhood-onset osteoporosis with few other syndromic features and cause **bone mineral density quantitative trait locus 18** (BMND18) (#300910). It is X-linked dominant so males are more severely affected than females. The function of PLS3 is still unknown but there is some suggestion that it may be important in the bones' response to mechanical strain [159].

NOTCH Signalling

Hajdu-Cheney syndrome (HJCYS) (#102500) is characterized by acro-osteolysis, severe osteoporosis, severe short stature and craniofacial features as well as other non-skeletal manifestations. Whole exome sequencing revealed mutations in *NOTCH 2* (*600275) that is important in early skeletal patterning and homeostasis. A characteristic radiological sign is acro-osteolysis but trans-iliac bone biopsies have shown a variable picture. HJCYS is a multisystem disorder and needs management as such.

Primary osteoporosis may be associated with mutations in any of the genetic causes of the **Ehlers–Danlos** (EDS) or **EDS-like syndromes,** as well as autosomal dominant conditions such as **Marfan syndrome** (#154700) (*FBN1*; *134797), **Cleidocranial dysostosis** (CCD; #119600) (*RUNX2*; *600211), **pseudoachondroplasia** (PSACH; #177170) (*COMP*; *600310), **22q-duplication syndrome** (#253250) (*TBX1*; *602054), **hyper IgE (Job) syndrome** (HIES; #147060) (*STAT3*; *102582) and autosomal recessive causes such as **geroderma-osteodysplasticum** (GO; #231070) (*GORAB*; *607983), **cutis laxa with progeroid features** (ARCL2B; #612940) (*PYCR1*; *179035), **mulibrey nanism** (#253250) (*TRIM37*; *605073), **polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (Nasu–Hakola disease)** (PLOSL/NHD; #221770) (*TYROBP*; *604142 or *TREM2*; *605086) and the **linkeropathies**, which are involved in bone protein processing (#245600, #615349 or #130070) (*B3GAT3*; *606374, *B4GALT7*; *615291 and *B3GALT6*; *604327, respectively).

Secondary Osteoporoses

The skeleton is a highly sensitive and responsive organ that plays an important role in energy metabolism, haemopoiesis and mineral homeostasis and as a scaffold. Mechanical loading of the skeleton is important in attaining and maintaining bone strength by enhancing mineralization and morphology. The skeleton can be compromised in times of acute and chronic illness, in haematological conditions, metabolic disorders, pubertal delay, during periods of reduced loading such as immobility and secondary to pharmacological intervention such as long-term steroid use. The key to managing secondary osteoporosis is early identification, prevention and intervention.

Neuromuscular Disorders The most important neuromuscular disorders where monitoring of bone health is important are **Duchenne muscular disorders** (DMD) (#310200) and **cerebral palsy**. Patients with DMD have multiple risk factors such as muscle weakness, long-term high-dose steroids and pubertal delay. The greatest decrease in bone density is when ambulation decreases. However, many boys lose all independent ambulation after a fracture, which then leads to a greater loss in bone density. Bisphosphonates are of benefit and the use of zoledronic acid has been of particular benefit due to

reduced time spent in hospital for treatment compared to pamidronate. Prophylactic treatment with bisphosphonates to prevent bone loss is controversial.

Children with CP have long slender bones with thin cortices and the morphology of the bones is compromised due to the lack of muscle loading on the skeleton. A 4–12% fracture rate has been reported [160], the majority being low impact fractures of the distal femur or proximal tibia. These children often do not have regular skeletal health monitoring so the number of vertebral fractures may be underestimated. Children with CP have low bone turnover. Bisphosphonates therefore do not seem to be the ideal therapy for this as it is anti-catabolic but, as anabolic agents have not been used in children, there have been small trials of bisphosphonate use in children that demonstrate an increase in bone mineral density. Bisphosphonate increases bone mass, which is augmented by growth, so it is important to treat these children before growth is complete.

Childhood Leukaemia Skeletal abnormalities are present in up to 75% of children at diagnosis of **acute lymphoblastic leukaemia** (ALL) with 16% having evidence of a vertebral compression fracture [161]. 60% of children with a BMD Z score <–2 sustain at least one fracture during the 3 years following diagnosis. For the majority of these children, there is resolution of skeletal morbidity with therapy and cure but there are children left with permanent skeletal deformities [162] and the skeletal health in children with ALL should be monitored. Management of fragility fractures is not clear since there is potential for remodelling and healing. Bisphosphonates should be reserved for the treatment of pain, functional impairment or where there is little growth potential left in older patients.

Hypogonadism and Delayed Puberty Lack of or resistance to oestrogen and testosterone impair bone strength in adolescents and adults. Oestrogen is of greater importance and deficiency leads to a high turnover osteoporosis and increased fracture risk, as seen in the postmenopausal population. In young adults, oestrogen deficiency occurs in **Turner syndrome**, following radiation and chemotherapy, **anorexia nervosa** and **hypopituitarism**. Lack of testosterone adversely affects muscle strength and is associated with impaired periosteal bone apposition. Puberty should be monitored in DMD and CP and, if necessary, hormone replacement instituted.

Anorexia Nervosa The low body mass is associated with increased fractures. In athletes there is a characteristic triad of low energy intake, menstrual dysfunction and low bone density and the fracture prevalence can be as high as 31% in adolescent girls [163]. Mainstay of management consists of psychological therapy in conjunction with a nutritional and behavioural programme.

Chronic Inflammatory Diseases Chronic inflammation is associated with increased resorption and osteoporosis. Many disorders such as rheumatic or **inflammatory bowel disease**, **cystic fibrosis** and **epidermolysis bullosa** have been described. Many of these are also associated with immobility, delayed puberty and the use of steroids. These different comorbidities adversely affect bone health but, if there have been recurrent fragility fractures or vertebral compression fractures, treatment with bisphosphonates should be considered.

Treatment of the Osteoporoses

Treatment of primary osteoporosis depends on the severity in any individual. For more severe forms, a multidisciplinary approach is taken by a team that might include a pediatrician, pediatric endocrinologist, physiotherapist, occupational therapist, orthopaedic surgeon, dentist, specialist nurse and social worker [164]. Access to a geneticist is advisable and ophthalmology and audiology may be required. It is inevitable, therefore, that good quality treatment will be undertaken only in specialist centres with a particular interest in the condition.

Apart from the routine treatment of fractures, surgery is often required to correct bone deformity. This may involve surgical fracture of bones with insertion of rods to maintain the integrity of the bones. Physiotherapy and other measures to increase mobility are essential as immobility has a detrimental effect on bone health, which can be reversed by enabling the patient to be more mobile. The mainstay of medical treatment is intravenous or, more rarely, oral bisphosphonates, which have a dramatic effect on diminishing bone pain, improving bone density, decreasing fracture rates and enabling mobilization of previously immobile patients.

Secondary osteoporosis is best treated by removing the cause of the osteoporosis. If the patient can be weaned off steroids or adequately mobilized, bone density may improve. If this proves impossible (e.g. in paraplegic patients), the use of bone-sparing drugs such as the bisphosphonates may be of value.

Diseases Characterized by Increased Bone Density

The conditions associated with increased bone density occur as a result of a mismatch between osteoblast and osteoclast function that favours the former. The osteoclasts may be poorly formed (osteoclast poor) or poorly functioning (osteoclast rich). This group of conditions includes the **osteopetroses**, **pyknodysostosis**, **van Buchem disease** and **sclerosteosis** (Table 10.6).

Table 10.6 Genetic disorders of altered bone mass. The metabolic abnormalities, together with the OMIM numbers of the conditions and their genes and modes of inheritance are shown.

Diagnosis	OMIM	Gene location	Gene	OMIM gene number	Inheritance
Conditions associated with low bone mass					
Primary osteoporosis					
Osteogenesis imperfecta (see Table 10.5)					
Other primary causes of osteoporosis					
Bruck syndrome type 1	#259450	17p12	*FKBP10*	607063	AR
Bruck syndrome type 2	#609220	3q24	*PLOD2*	601856	AR
Gnathodiaphyseal dysplasia	#166260	11p14.3	*ANO5*	608662	AD
Cole-Carpenter syndrome	112240	3p22, 3p24.1-p22	*CRTAP*	605497	
Idiopathic juvenile osteoporosis	259750	10q21.1	*DKK-1*	605189	AD
	259750	11q13.4	*LRP5*	603506	AD
	259750	12q13	*WNT1*	164820	AD
	259750	1q42	*WNT3A*	606359	AD
Other conditions associated with low bone mass					
Ehlers–Danlos syndrome					AD
Marfan syndrome	#154700	15q21.1	*FBN1*	134797	AD
Osteoporosis pseudoglioma syndrome	#259770	11q13	*LRP5*	603506	AR
Idiopathic juvenile osteoporosis					
Juvenile Paget disease	#239000	8q24.12	*TNFRSF11B* (Osteoprotegerin)	602634	AR
Geroderma osteodysplasticum	#231070	1q24.2	*GORAB*	607983	AR
Cutis laxa with progeroid features	#612940	17q25.3	*PYCR1*	179035	AR
Cleidocranial dysostosis	#119600	6p21.1	*RUNX2*	600211	AD
Pseudoachondroplasia	#177170	19p13.11	*COMP*	600310	AD
PTHLH duplication					
Hajdu-Cheney syndrome	#102500	1p12-p13	*NOTCH2*	600275	AD
Mulibrey nanism	#253250	17q22-23	*TRIM37*	605073	AR
Chromosome 22q.11 duplication syndrome	#608363	22q11.2	*TBX1*	602054	AD/sporadic
Hyper IgE syndrome	#147060	17q21.2	*STAT3*	102582	AD
Polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (Nasu-Hakola disease)	#221770	19q13.12	*TYROBP (DAP12)*	604142	AR
Polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (Nasu-Hakola disease)	#221770	6p21.1	*TREM2*	605086	AR

Linkeropathies					
Multiple joint dislocations, short stature, craniofacial dysmorphism and congenital heart defects (formerly AR Larsen syndrome)	#245600	11q12.3	*B3GAT3*	606374	AR
Galactosyl transferase 1 deficiency (Ehlers–Danlos progeroid type 1)	#615349	1p36.33	*B4GALT7*	615291	AR
Galactosyl transferase 2 deficiency (Ehlers–Danlos progeroid type 2)	#130070	5q35.3	*B3GALT6*	604327	AR
Secondary causes of altered bone mass					
Endocrine disorders, e.g. Cushing syndrome					
Iatrogenic causes, e.g. steroid therapy					
Nutritional disorders					
Chronic diseases, e.g. beta-thalassaemia					
Malignancies, e.g. leukaemia					
Disuse, e.g. following immobilization					
Other specified disorders	#320200	Xp21.2-p21.1	*Dystrophin*	*300377	XLR
Other unspecified disorders					
Other conditions associated with low bone mass, specified					
Other conditions associated with low bone mass, unspecified					
Conditions associated with high bone mass					
Osteopetrosis					
Osteoclast-rich osteopetrosis					
Autosomal recessive 1 (OPTB1) (malignant)	#259700	11q13.4-q13.5	*TCIRG1* subunit	604592	AR
Autosomal recessive 3 (OPTB3) with renal tubular acidosis (intermediate)	#259730	8q22	*CA2*	611492	AR
Autosomal recessive 4 (OPTB4) (malignant/intermediate)	#611490	16p13	*CLCN7*	602727	AR
Autosomal recessive 5 (OPTB5) (malignant)	#259720	6q21	*OSTM1*	607649	AR
Autosomal recessive 6 (OPTB6) (intermediate)	#611497	17q21.3	*PLEKHM1*	611466	AR
Autosomal recessive 8 (OPTB8)	#615085	7p15.2	*SNX10*	614780	AR
Autosomal dominant 1 (OPTA1)/LRP5 activation (van Buchem disease type 2)	#607636	11q13.2	*LRP5*	603506	AD
Autosomal dominant 2 (OPTA2) (Albers Schönberg disease) (benign)	#166600	16p13	*CLCN7*	602727	AD
Osteoclast-poor osteopetrosis					
Autosomal recessive 2 (OPTB2) (benign)	#259710	13q14	*TNFSF11* (RANK Ligand)	602642	AR
Autosomal recessive 7 (OPTB7) (malignant)	#612301	18q22.1	*TNFRSF11A* (RANK)	603499	AR

(*Continued*)

Table 10.6 (Continued)

Diagnosis	OMIM	Gene location	Gene	OMIM gene number	Inheritance
Other diseases associated with high bone mass					
Sclerosteosis type 1	#269500	17q21.31	*SOST*	605740	AR
Hyperostosis corticalis generalisata (van Buchem disease type 1)	#239100	17q21.31	*SOST*	605740	AR
Endosteal hyperostosis, osteosclerosis (Worth disease)	#144750	11q13.2	*LRP5*	603506	AD
Craniodiaphyseal dysplasia	#122860	17q21.31	*SOST*	605740	AD
Sclerosteosis type 2	#614305	11p11.2	*LRP4*	604270	AD/AR
Pycnodysostosis	#265800	1q21	*CTSK*	601105	AR
Osteopathia striata with cranial sclerosis	#300373	Xq11.2	*WTX* or *AMER1* or *FAM123B*	300647	XLD
Osteopoikilosis	#166700	12q14.3	*LEMD3*	607844	AD
Buschke-Ollendorf syndrome	#166700	12q14.3	*LEMD3*	607844	AD
Melorheostosis	%155950	12q14.3	*?LEMD3*	607844	AD
Pachydermoperiostosis type 1	#259100	4q31.4	*HPGD*	601688	AR
Cranioosteoarthopathy	#259100	4q31.4	*HPGD*	601688	AR
Pachydermoperiostosis type 2	#614441	3q22.1-q22.2	*SLCO2A1*	601460	AR
Primary hypertrophic osteoarthropathy	%167100				AD/AR
Raine syndrome (lethal osteosclerotic bone dysplasia)	#259775	7p22.3	*FAM20C*	611061	AR
Chondrocalcinosis type 2	#118600	5p15.2	*ANKH*	605145	AD
Craniometaphyseal dysplasia	#123000	5p15.2	*ANKH*	605145	AD
Craniometaphyseal dysplasia	#218400	6q22.31	*GJA1*	121014	AR
Anhidrotic ectodermal dysplasia with immunodeficiency, osteopetrosis and lymphoedema	#300301	Xq28	*IKBGK*	300248v	XLR
Inherited inflammatory conditions of bone					
Caffey's disease	#114000	17q21.33	*COL1A1*	120150	AD
Camurati-Engelmann disease	#131300	4p16.3	*TGFB1*	602104	AD
Cherubism	#118400	19q13.2	*SH3BP2*	190180	AD

Osteopetrosis (OPT)

Autosomal recessive osteopetrosis (ARO), also known as malignant infantile osteopetrosis, is a heterogeneous group of disorders divided mechanistically into osteoclast dysfunction (osteoclast-rich ARO) or deficiency in osteoclast number (osteoclast-poor ARO). These groups present with early-onset symptoms and often can be diagnosed radiologically before birth. Bone overgrowth results in apparent macrocephaly, with progressive blindness, deafness and anaemia because of encroachment on cranial nerves and bone marrow. Hepatosplenomegaly from extra-medullary haematopoiesis is present and leukoerythroblastic anaemia should raise the possibility of OPT. Despite increased bone density, the bones are more brittle and fractures may occur. Hypocalcaemia with evidence of rickets may also be present, particularly in the malignant forms. Left untreated this disorder can be fatal so early diagnosisis is important. The mainstay of treatment is bone marrow transplant (BMT) and eligibility can rest on the clinical severity and genetic mutation.

Autosomal recessive osteopetrosis type 1 (OPTB1) (#259700) is the most common osteoclast-rich malignant infantile form of OPT and is caused by mutations in the *T-cell immune regulator subunit of the vacuolar proton pump* (*TCIRG1 subunit*) (*604592). TCIRG is the protein involved in transporting hydrogen ions into the resorption lacunae to acidify and resorb bone (Figure 10.7). BMT is the mainstay of treatment but should be performed before nerve compression supervenes.

Autosomal recessive osteopetrosis type 2 (OPTB2) (#259710) is a relatively mild osteoclast-poor form of OPT characterized by prognathism, genu valgum and an increased fracture tendency. Anaemia and hepatosplenomegaly are present in some cases. The underlying cause is mutations in the *TNFS11* gene (*602642) that codes for RANKL (Figure 10.7). Osteoclasts are not transformed in normal numbers and bone resorption is therefore defective. It is not amenable to BMT as there is no intrinsic defect in osteoclast function.

Autosomal recessive osteopetrosis type 3 (OPTB3) (#259730) is a 'benign' form of OPT caused by mutations in the *carbonic anhydrase II* (*CA2*) gene (*611492). Like the vacuolar proton pump, it contributes to the development of the bone-resorbing acid environment of the osteoclast ruffled border and is therefore an osteoclast-rich form of the condition associated with mild nerve compression, particularly of the optic nerve, dental malocclusion, short stature and a degree of mental retardation. Mild anaemia may be present but tends to resolve with time. The CA2 deficiency also gives rise to a degree of renal tubular acidosis.

Autosomal recessive osteopetrosis type 4 (OPTB4) (#611490) is a malignant infantile form of OPT caused by mutations in the *chloride channel 7* (*CLCN7*) gene (*602727) responsible for transporting the chloride ions that, with the protons, provide the hydrochloric acid that dissolves bone mineral. The clinical features are similar to those of OPTB1. BMT may help.

Autosomal recessive osteopetrosis type 5 (OPTB5) (#259720) is very similar to OPTB4. It is another malignant infantile form of OPT. Mutations in *OSTM1* (*607649) result in an abnormal β-subunit of CLCN7 that also results in failure of osteoclast acidification.

Autosomal recessive osteopetrosis type 6 (OPTB6) (#611497) is a relatively mild form of OPT that presents with walking difficulties and leg pains. Initially patients have dense metaphyseal bands on X-ray but later develop an 'Erlenmeyer flask' appearance of the distal femora. Mutations in the pleckstrin gene (*PLEKHM1*) (*611466) involved in vacuolar transport in osteoclasts are responsible.

Autosomal recessive osteopetrosis type 7 (OPTB7) (#6123011) is caused by mutations in *TNFRSF11A* (RANK), the receptor on the osteoclast surface for RANKL. This causes an osteoclast-poor form of OPT but is amenable to treatment with BMT.

Autosomal recessive osteopetrosis type 8 (OPTB8) (#615085) is caused by mutations in *sorting nexin-10 (SNX10)* (*614780) and gives rise to a malignant form of osteoclast-rich OPT similar to OPTB1.

Autosomal dominant osteopetrosis type 1 (OPTA1), also known as **van Buchem disease type 2** or **Worth** disease, (#607634) is an abnormality of osteoblast formation that gives rise to endosteal hyperostosis that is caused by activating mutations in *LRP5*. In contrast to OPPG, these mutations result in an abnormal protein that cannot bind to, and therefore be inhibited by, Dickkopf (*605189) (Figure 10.6). As a result, LRP5 binds avidly to frizzled protein and overstimulates osteoblast activity. It is not strictly a form of OPT as there is no defect in osteoclast function but gives rise to decreased osteoclast numbers. Osteosclerosis is mainly seen in the skull vault while the spine is almost completely spared with no evidence of a 'bone within bone' appearance. These patients are not susceptible to fractures and both bone density and bone strength are increased.

Autosomal dominant osteopetrosis type 2 (OPTA2), **Albers-Schönberg** or **marble bone disease** (#166600) is another milder form of OPT caused by heterozygous mutations in *CLCN7*. Since chloride channels exist as dimers, it seems that a dominant negative effect is present to account for the abnormality. The condition can be differentiated from OPTA1 by the distribution of the osteosclerosis, which mainly affects the skull base, spine and pelvis. A 'bone within bone' appearance is seen in the vertebrae. Increased bone fragility and dental abscesses are prominent features.

Other Miscellaneous Conditions

Pycnodysostosis (PKND) (#265800) is caused by AR mutations in the gene coding for *cathepsin K (CTSK)* (*601105), which is synthesized by the osteoclasts and is responsible for resorption of bone matrix once the mineral has been removed. Extreme short stature, abnormalities of the skull vault, with delayed fusion of the sutures and acro-osteolysis of the maxilla and phalanges are prominent features. Increased bone fragility is present and stress fractures of the tibia, femur and lumbar and cervical vertebrae may occur. Bone overgrowth can sometimes lead to extra-medullary haematopoiesis similar to the more severe forms of OPT.

Sclerosteosis (SOST) (#269500) and **van Buchem disease type 1** (VBCH1) (#239100) are both caused by mutations in *SOST* (*605740). Sclerostin is normally produced by osteocytes and inhibits the action of BMPs, which stimulate osteoblast differentiation. Bone overgrowth results from unrestrained osteoblast transformation. SOST is the more severe disease with features of tall stature, increased body weight and overgrowth of the long bones and skull, the latter resulting in cranial nerve compression, which is often multiple. It is particularly common in the Afrikaner population of South Africa. VBCH1 is less severe and may result from mutations downstream of *SOST* itself within a proposed *SOST gene regulator*. It usually presents in late childhood or adolescence with osteosclerosis of the skull, which may be up to four times normal weight, mandible, long bones and ribs. Cranial nerve compression, particularly of the optic and auditory nerves, may occur.

Craniometaphyseal dysplasia (CMDD) (#123000), caused by mutations in *ANKH* (*605145), which is responsible for transporting pyrophosphate from the intracellular to the extracellular space in osteoclasts, particularly affects the craniofacial bones. Widening of the bridge of the nose together with the development of 'leonine' facies may result in cranial nerve compression. Increases in pyrophosphate concentrations inhibit bone resorption leading to bone overgrowth.

Paget disease of bone is a heterogeneous group of disorders characterized by increased bone turnover in one or more areas of bone, mainly in the axial skeleton. Most cases occur after the age of forty but **juvenile Paget disease type 5** (PDB5) (#239000), usually caused by mutations in the *TNFRSF11B* gene that encodes osteoprotegerin (*602643) [165], is an autosomal recessive condition of increased bone resorption caused by abnormal osteoclast function. This mutation normally exercises an inhibitory effect on RANKL to prevent overstimulation of osteoclasts via the RANK receptors on osteoclast precursors (Figure 10.7). Bone turnover is high and markers of bone turnover, such as alkaline phosphatase, NTX and hydroxyproline, are increased.

Patients present in early life with a large head. The long bones are bowed and greatly expanded and show coarse trabeculation on X-ray. The calvaria show marked thickening with isolated areas of increased density. Treatment with bisphosphonates should be of value but the most logical treatment is with recombinant osteoprotegerin, which has been used in adults with success [166]. There are no reports of its use in children. Denosumab, a monoclonal antibody inhibitor of RANKL, could also theoretically be of value.

Early-onset Paget disease type 2 (PDB2) (#602080) has a similar phenotype but presents later than PDB5, usually in the teens or early twenties. It is an autosomal dominant condition caused by mutations in *TNFRSF11A (RANK)*. Similar mutations also cause **familial expansile osteolysis** (FEO) (#174810), which mainly affects the appendicular skeleton.

Other forms of Paget disease are caused by mutations in *sequestosome* (*SQSTM1*) (*601530) (PDB3; (#167250), and *ZNF687* (*610568) (PDB6; #616833). Some of these patients are prone to giant cell tumours of bone or osteosarcoma.

Inherited Inflammatory Diseases of Bone

Caffey's disease (infantile cortical hyperostosis) (#114000) presents in infancy before the age of five months and may be present at birth or *in utero*. It is characterized by inflammation, mainly of the mandible and ribs, although other long bones can be affected. It usually settles by the age of two years although recurrences can occur for many years. Bone deformities are unusual. It is most commonly autosomal dominant, caused by a specific mutation in exon 42 of *COL1A1*. An acquired form is described in premature infants with cyanotic congenital heart disease who have been treated for long periods with prostaglandin E2 to maintain the patency of the ductus arteriosus prior to surgery.

Camurati–Engelmann disease (CED) (#131300) [167] is an autosomal dominant condition caused by mutations in *TGFB1* (*190180). It affects the diaphysis of long bones, particularly tibiae and humeri but may later spread to other long bones. It is characteristically symmetrical and causes pain and swelling associated with muscle weakness, waddling gait and easy fatiguability. Treatment with prednisone is usually effective in controlling the pain and losartan has been shown to be effective.

Cherubism (#118400) is an autosomal dominant condition caused by mutations in *SH3BP2* (*602104). It usually appears in early childhood and progresses until the mid-teens with swelling and pain, particularly of the mandibles, maxillae and medial ends of the ribs. It can

sometimes be confused with Caffey disease but the symptoms are self-limiting.

Raine syndrome (#259775), a consequence of an autosomal recessive mutation in *FAM20C* (*611061), is an aggressive sclerotic bone condition with characteristic facial features of a narrow prominent forehead, proptosis and midfacial hypoplasia. There is periosteal bone formation along the long bones and intracranial calcification. It normally results in neonatal death but there have been some survivors.

Disorders of Phosphate Metabolism

The key to the control of phosphate metabolism is FGF23 and the mechanisms by which the conditions associated with altered phosphate metabolism arise can be divided into those in which FGF23 is raised and those in which it is too low. Plasma phosphate is usually, though not always, low in those with raised FGF23 and *vice versa*. There is a group of conditions in which plasma phosphate is normal but which also cause soft tissue calcification (Table 10.6).

Hypophosphataemic Rickets Associated with Raised FGF23

X-linked dominant hypophosphataemic (vitamin D-resistant) rickets (XLH) (#307800) caused by mutations in *PHEX* (*300550) located on the X-chromosome is the commonest form of hypophosphataemic rickets. Affected individuals present within the first or second year of life with bowed legs and signs of rickets. There may be a family history. Boys are more severely affected than girls because of the absence of a normal allele on the second X-chromosome. The phenotype in females is very variable and not necessarily consistent within families – maternal carriers may be unaware that they have it until tested following diagnosis in a child.

The clinical signs of rickets are similar to those of vitamin D-related rickets although often without muscle weakness and pain. Dental abscesses may occur because of dentine defects. Widening of the wrists and knees, bowing of the legs and a 'rickety rosary' are present. Radiological signs showing a rather coarser trabeculation than those of vitamin D-related rickets may suggest the diagnosis. Growth may be impaired. Urinary phosphate excretion is raised and plasma phosphate low. Plasma calcium is normal in the untreated state and alkaline phosphatase and FGF23 are raised.

Treatment consists of a combination of oral phosphate supplements with alfacalcidol or, where this is not available, calcitriol. The vitamin D analogue is required to counteract the suppressive effect of FGF23 on 1α-hydroxylase activity and to prevent hypocalcaemia. Unfortunately phosphate supplements need to be given four or five times a day to be effective because urinary excretion is so rapid. The dose of phosphate is often limited by diarrhoea. Putting a day's supply of phosphate into a bottle and asking the child to sip from it at regular intervals may be a successful way round this problem. Getting the balance right between these two treatments and preventing hypocalcaemia while obtaining healing of the rickets can be difficult. Excess phosphate can result in hyperparathyroidism, while too much vitamin D is likely to cause hypercalciuria and nephrocalcinosis. GH has been used with some effect to improve growth and it may have a temporarily beneficial effect on phosphate excretion [168, 169]. A more logical treatment is to use a monoclonal antibody against FGF23 and preliminary studies have been encouraging. This treatment, Burosumab, has been been proven to be efficacious, and has now been licensed for use in growing children over the age of one year. Monitoring treatment requires regular measurements of calcium, phosphate, PTH, urinary calcium/creatinine ratio and alkaline phosphatase in plasma and measurements of FGF23 may also be useful. X-rays may show healing of the rickets but it is important to understand that, unlike vitamin D-related rickets, the bones never return to normal and biopsy evidence of osteomalacia persists, however effective the treatment. Renal ultrasound should be performed annually to screen for nephrocalcinosis. Orthopaedic intervention may be required if bone deformity becomes significant, particularly if it impairs mobility. Growth and bone deformities often improve with adequate treatment.

ADHR (#193100) is clinically and biochemically very similar to, though milder than, XLH, although the diagnosis is often not made until a later age and may not become apparent until adolescence. It is caused by mutations in *FGF23* (#241520), which prevent the natural cleavage of the molecule, thus maintaining higher than normal concentrations of FGF23. It is much rarer than XLH but treatment is similar; FGF23 production is inversely related to serum iron and it is important to treat iron deficiency. The hypophosphataemia occasionally resolves with age.

Autosomal recessive hypophosphataemic rickets type I (ARHR1) (#241520) is a rare form of hypophosphataemic rickets that is also clinically and biochemically similar to XLH and ADHR but is caused by mutations in *DMP1* (*600980). DMP1 is secreted by osteocytes and contains the ASARM motif that is a specific substrate for PHEX, which, if unbound, cannot institute cleavage of FGF23, which is therefore elevated. Treatment is the same as that for XLH and ADHR.

Autosomal recessive hypophosphataemic rickets type II (ARHR2) (#613312) is another rare form of hypophosphataemic rickets caused by homozygous mutations in *ENPP1* (*173335). This most commonly causes **generalized arterial calcification of infancy** (GACI) (#208000), which is fatal in 85% of cases. Should patients survive, they get hypophosphataemic rickets because of inhibition of FGF23 cleavage by lack of ENPP1. GACI can also be caused by mutations in *ABCC6*, although this is not accompanied by hypophosphataemia [170].

Polyostotic fibrous dysplasia (PFD) and **McCune–Albright syndrome** (MAS) (#174800) is caused by mosaicism for activating somatic mutations in *GNAS1* (*139320). Fibrous dysplastic lesions can occur in any bone and may be limited or widespread. The accompanying activation of Gsα-dependent hormone stimulation and the characteristic 'coast of Maine' pigmented skin lesions are described elsewhere. Dysplastic lesions may cause pathological fractures but may also cause hypophosphataemic rickets and osteomalacia because of increased concentrations of FGF23, which is secreted by the bone lesions.

Bisphosphonates are sometimes used as specific treatment for PFD, particularly for the accompanying bone pain [171]. The bone lesions do not return to normal but pain relief is sometimes considerable. Since there is no specific abnormality of the otherwise normal bone, treatment is aimed at symptom relief rather than being given regularly. If hypophosphataemia is present, oral phosphate supplements should be given to prevent worsening hypophosphataemia causing acute cardiovascular disturbances. Theoretically, the FGF23 monoclonal antibody, burosumab, should be of value in treating the rickets.

Tumour-induced osteomalacia (TIO) Occasionally tumours, usually of mesenchymal origin and non-malignant, are associated with rickets and osteomalacia, which is of hypophosphataemic origin. The mechanism is not clear but probably results from the production of humoral factors by the tumour such as MEPE, which contains the ASARM motif that competes for binding sites on PHEX but does not contain the RGD motif that allows it to bind to integrins on the cell surface. FGF23 therefore remains high. The condition is best treated by tumour removal although they may be difficult to locate. Other tumours secrete high concentrations of FGF23.

Epidermal nevi Several epidermal naevus syndromes, such as **phakomatosis pigmentokeratotica** and **Schimmelpenning-Feuerstein-Mims syndrome** (#163200), have been reported to be associated with hypophosphataemic rickets [172–174]. The mechanism is uncertain but is thought to be similar to that of TIO and FGF23 is raised. Effective removal of the naevi is sufficient to cure the osteomalacia although this can prove problematic, especially if the lesions are deep seated.

One specific cause is the **congenital melanotic nevus syndrome** (CMNS; #137550), which is caused by activating mutations in *NRAS* (*164790), part of the RAS/MAPK pathway signalling molecules that play a role in cell growth, differentiation and survival. The mutations are somatic. Widespread melanotic lesions are present at birth and there may be CNS involvement.

Hypophosphataemic Rickets Associated with Low FGF23

Hereditary hypophosphataemia with hypercalciuria (HHRH) (#241530), unlike XLH, ADHR and ARHR, is not related to abnormalities in FGF23. It is an autosomal recessive condition caused by a defect in the sodium phosphate co-transporter of the renal tubule (*609826) caused by mutations in *SLC34A3*, which results directly in increased phosphate excretion, although heterozygous carriers show some intermediate effects on calcium and phosphate transport between homozygotes and normal individuals. As a consequence, FGF23 is low and $1\alpha,25(OH)_2D$ appropriately raised as a result of the hypophosphataemia and the lack of inhibition by FGF23. Urinary calcium excretion is therefore elevated with the subsequent risk of nephrocalcinosis. Treatment consists of phosphate supplements that usually correct the biochemical abnormalities apart from the low renal phosphate threshold. Vitamin D analogues should not be used because of the tendency to increase urinary calcium excretion.

Fanconi syndrome is a generic term for a number of proximal renal tubular disorders that, in its full manifestation, consists of bicarbonaturia, glycosuria, aminoaciduria and phosphaturia [175]. It has a wide variety of acquired and genetic causes, the commonest of which is **cystinosis** (CTNS) (#219800). Rickets may be the first manifestation but chronic renal failure may supervene without appropriate treatment. In this case rickets usually disappears because phosphate excretion diminishes as a result of the renal failure. Table 10.3 gives details of the principal genetic conditions as well as details of other renal tubular disorders associated with hypercalciuria and with nephrocalcinosis. Specific treatment of the rickets consists of correcting the acidosis (which may require large quantities of bicarbonate) and reversing the hypophosphataemia.

Acquired hypophosphataemia can occasionally result from low gastrointestinal or dietary intake although phosphate absorption is very efficient and this is unusual in the absence of gastrointestinal disease. Recovery from fasting either actual or effective (e.g. diabetic ketoacidosis) can result in hypophosphataemia due to the repartitioning of phosphate from extracellular to intracellular spaces in the **refeeding syndrome**. Intravenous supplementation may occasionally be required if cardiac effects are seen.

Soft Tissue Calcification Disorders

Hyperphosphataemic familial tumoural calcinosis (HFTC) (#211900) is a rare autosomal recessive condition characterized by progressive deposition of painful calcific nodules in periarticular spaces and soft tissues including the skin. The nodules consist of basic calcium phosphate and should be distinguished from extra-skeletal ossification. The underlying biochemical defect is hyperphosphataemia secondary to inactive or ineffective FGF23 and can be caused either by inactivating mutations of *FGF23* itself (*605380) (HFTC2) or by mutations in *GALNT3* (*601756) (HFTC1), which prevents FGF23 from being processed properly, or by mutations in *Klotho* (+604824) (HFTC3) that prevent FGFR1 from being able

to act as a suitable receptor for FGF23 (Figure 10.5). In the first two conditions, active FGF23 is low while in the third it is elevated. This results in suppression of 1-α-hydroxylase activity, hypocalcaemia and secondary hyperparathyroidism, sometimes necessitating parathyroidectomy. In all cases TRP is increased and calcium phosphate deposition occurs. Angioid streaks of the retina, similar to those of **pseudoxanthoma elasticum** (#264800), are usually present.

Treatment is difficult but some success has been achieved with a low phosphate diet and phosphate-binding agents such as aluminium hydroxide. Theoretically the newer phosphate-binding agent, sevelamer, should also be effective without running the risk of aluminium toxicity [176]. Acetazolamide has also been shown to be useful [176]. Some success has also been described with the use of topical sodium thiosulphate [177]. The sodium is exchanged for calcium whose thiosulphate is two hundred times more soluble than the phosphate. Surgery may be needed to remove troublesome lesions but they often recur [176].

Hyperostosis-hyperphosphaemia syndrome (HHS) (#610233) overlaps HFTC and is also caused by mutations in *GALNT3*. It is characterized by recurrent transient episodes of swelling and pain in long bones accompanied by radiological evidence of periosteal reactions. Skin and other soft tissues are not involved in the same way as in HFTC.

Certain mutations of *FGFR1c* (*136350) are associated with soft tissue calcification. **Hartsfield syndrome** (#615465) not only is notable for the multiple congenital abnormalities but also has soft tissue calcification as a feature.

Other Causes of Soft Tissue Calcification

Normophosphataemic familial tumoural calcinosis (NFTC) (#610455) is similar to HFTC but is not accompanied by hyperphosphataemia. It is caused by inactivating mutations in *SAMD9* (*610456), which is involved in fibromatous tumour suppression.

Progressive osseous heteroplasia (POF) (#166350) is caused by heterozygous paternally inherited inactivating mutations of *GNAS1* (*139320). In contrast to HFTC and HHS, the lesions in this condition consist of bony spicules that contain normal membranous bone structures. Osteoma cutis, hard swellings of the skin, occur initially, often in very young children. These later become ossified and visible radiologically. There is some overlap between this condition and **AHO** (#103580) in which heterotopic ossification (osteoma cutis) can also occur caused by similar mutations.

Fibrodysplasia ossificans progressiva (FOP) (#135100) is an autosomal dominant condition characterized by congenital malformation of the big toe and progressive heterotopic endochondral ossification in skeletal muscle, ligaments and tendons. It is important to make an early diagnosis as patients develop painful soft tissue swellings that progress to heterotopic mature bone formation within skeletal muscle and soft tissues. Patients with FOP have a progressive disorder and have commonly lost all mobility by the third decade and suffer from restrictive respiratory compromise. FOP is caused by a mutation leading to an overactive *ACRV1/ALK2* receptor, part of the BMP pathway. A phase 2 clinical trial is currently being conducted, involving palovarotene, which is a retinoic acid receptor gamma (RARγ) agonist acting to inhibit secondary messengers in the BMP pathway.

Other rare inherited conditions with high bone density or soft tissue calcification phenotypes include **Rothmund–Thomson syndromes** (#268400) **types 1 and 2** (*RECQL4*; *603780), **poikiloderma with congenital neutropaenia** (#604170) (*C16orf57*; *613276) and **pulmonary microlithiasis** (#265100) (*SLC34A2*; *604217).

Acquired hyperphosphataemia can occasionally follow excessive dietary intake, acute release of phosphate from cells during rhabdomyolysis, tumour lysis of intravenous haemolysis or excessive bone resorption in high turnover diseases.

Drugs Used in the Treatment of Disorders of Calcium and Bone Metabolism

Vitamin D

The normal requirement for vitamin D in individuals not exposed to adequate sunshine is 400–800 IU (10–20 μg daily). Vitamin D is the treatment of choice for vitamin D deficiency. It can be given either as colecalciferol or as ergocalciferol. The former is probably slightly more effective but, in practice, there is little to choose between them. In the presence of deficiency, it can be given as a daily supplement (1,500–10,000 units daily depending on age) and should be given for a total of 3 months in the first instance. If there are concerns about compliance or a problem of malabsorption, larger single doses can be given, a regimen known as stosstherapy. Some authors have recommended this as the treatment of choice [178]. It can be given in divided doses orally during 1 day or as a single intramuscular dose. 150,000–600,000 units are given every 3 months, depending on age. A wide variety of preparations containing either vitamin D in combination with calcium or as a multivitamin preparation is available and varies from country to country.

Vitamin D Metabolites

1α-hydroxycholecalciferol (alfacalcidol) is vitamin D_3 that has been hydroxylated in the 1-position but not in

the 25-position. It is active orally but, once absorbed, has to be converted to $1,25(OH)_2D_3$ in the liver. It has a half-life of around 30–35 hours, which makes it ideal to be given daily. It is the treatment of choice in hypoparathyroidism, hypophosphataemic rickets, 1α-hydroxylase deficiency rickets and chronic renal failure, although in the latter vitamin D deficiency as a cause of raised PTH must be excluded and treated first [111]. The usual dose requirement is 30–50 ng/kg/day and treatment must be monitored to make sure that hypercalcaemia and/or hypercalciuria do not supervene. Higher doses may be needed initially until plasma calcium is stabilized. In hypoparathyroidism plasma calcium may need to be maintained at the lower end of the normal range or even lower, as long as the patient remains asymptomatic, particularly when activating mutations of *CaSR* are responsible, in order to prevent hypercalciuria.

Unfortunately, alfacalcidol is not available in some countries, including the USA, and calcitriol is usually used instead. 1α-hydroxyergocalciferol (Hectorol®) is available in the USA and licenced for use in chronic renal failure. It has one-half to two-thirds of the potency of alfacalcidol.

1α,25-dihydroxycholecalciferol (calcitriol) is the fully active metabolite of vitamin D. Because it does not need to be metabolized, it has a half-life of only 5–6 hours and therefore needs to be given at least twice and preferably 3 times a day. Its principal value is in situations where 25-hydroxylation of vitamin D is impaired such as in chronic liver disease and in BDP especially where there is a coexistent hepatitis. In countries where alfacalcidol is not available, calcitriol is usually used. The usual dose for maintenance is 15–30 ng/kg/day.

Paricalcitol (Zemplar®) is 19-nor calcitriol and has a preferential effect on the VDR in the kidney and less effect in the gut. It is sometimes used instead of alfacalcidol or calcitriol in chronic renal disease for this reason.

Teriparatide (Forsteo®)

This is synthetic PTH 1–34 and has recently become available for the treatment of postmenopausal osteoporosis and has occasionally been used to treat hypoparathyroidism in adults. It is not licenced for use in children and there are only a few short-term studies of its use in pediatric patients with hypoparathyroidism. It is effective in raising plasma calcium and may have a specific benefit in patients in whom hypercalciuria and nephrocalcinosis is a particular problem, such as in ADH [179] in which continuous subcutaneous infusion using an insulin pump has proved effective. There are no long-term safety or efficacy studies in children and the manufacturers have raised concerns about the possibility of developing osteosarcomas with long-term treatment although increasing use of this product has not confirmed these fears.

The Bisphosphonates

The bisphosphonates are analogues of pyrophosphate in which the central oxygen atom is replaced with carbon, the tetravalent nature of which allows the addition of extra residues on the 'spare' side chains, designated R1 and R2. In most bisphosphonates, R1 is a hydroxyl group that allows it to bind with pyrophosphate. The variety of the R2 residues determines the properties of the bisphosphonates and much work has been devoted to developing products that have a differential effect on bone accretion and resorption. The first generation of bisphosphonates, e.g. etidronate and clodronate, showed some of this differential but care has to be exercised in ensuring that they do not cause a mineralization defect and the later generations of drugs not only are much more potent (zoledronate is 10,000 times more potent than etidronate) but also have a greater effect on osteoclasts than on osteoblasts.

The mode of action has been well reviewed by Shaw and Bishop [180] and by Russell [181]. The first generation bisphosphonates act by forming acyclic analogues of ATP that are cytotoxic and lead to cell apoptosis. All of the later second to fourth generation bisphosphonates contain an amino group and act by inhibiting farnesyl diphosphate synthase that, via the mevalonic acid pathway, inhibits protein prenylation (transfer of fatty acid chains) into intracellular proteins that are unable to be incorporated normally into cell membranes [182]. This disrupts cell function and leads to apoptosis. The effect is temporary and, after a few weeks, the osteoclasts recover and resume their functions. Each episode of treatment can be identified radiologically by the presence of dense bands (Zebra lines) across the metaphyses and, as growth slows, these bands become closer together until they eventually fuse.

The greatest experience of bisphosphonate use in children is with pamidronate. The first report of any significant numbers of patients treated was in 1998 [183], which demonstrated in thirty patients the benefits of reduced bone pain and fracture frequency and increased bone strength and density. Pamidronate, usually given by intravenous infusion in a dose of 1 mg/kg daily for 3 days every 3–4 months has become standard for children with moderate to severe OI although the newer bisphosphonate, zoledronate, is being increasingly used. Bisphosphonates can be given orally but are poorly absorbed because of their highly polar nature. They should be given at least 2 hours away from food. Trials of oral alendronate or olpadronate have proved disappointing and the most potent, risedronate, may be helpful in maintenance and for pain relief in PFD.

The indications for the use of bisphosphonates in children are:

- Generalized osteoporotic conditions, such as OI and OPPG, as well as the secondary osteoporoses.

- Hypercalcaemic conditions such as those caused by immobilization, malignancy or hyperparathyroidism. These patients are usually very sensitive to treatment and may respond successfully to as little as a single dose of 0.5 mg/kg.
- Soft tissue calcification. There are no large series reporting the effects of bisphosphonates in these conditions but several case reports have demonstrated their value in a variety of conditions including dermatomyositis, fibrodysplasia ossificans progressiva, scleroderma and infantile arterial calcification.
- Miscellaneous conditions, particularly polyostotic fibrous dysplasia. The effects here are principally to reduce pain and the treatment has little effect on the bone lesions. Since the aim is not principally to increase bone density, treatment is usually given when the patient's symptoms demand it rather than on a regular basis.

Bisphosphonates are accompanied by a number of side effects:

- Acute phase reaction occurs only with the amino-bisphosphonates and is accompanied by fever, aches and pains and sometimes vomiting, which respond well to simple analgesics and antipyretics. They usually only occur during the first cycle of treatment.
- Hypocalcaemia and hypophosphataemia usually occur to a certain extent but this does not cause problems unless exaggerated by the presence of vitamin D deficiency. Patients should always be screened for this and, if necessary, treated prior to initiation of bisphosphonates.
- Oesophagitis. Some of the oral bisphosphonates, particularly alendronate and olpadronate, are reported to cause oesophagitis. This seems to be less of a problem in children than in adults and less so with risedronate.
- Osteonecrosis of the jaw has been reported in adults but there are no reports of its occurrence in children despite surveys of large numbers of treated children specifically looking for the problem.
- Osteopetrosis and undertubulation. There is one report of a child treated for non-specific bone pain with pamidronate at monthly intervals [184]. He developed dense bones, similar to those seen in osteopetrosis, and undertubulation, which results from failure of remodelling of the long bones. All known causes of osteopetrosis were excluded. The bone abnormalities persisted even after treatment had been stopped for 18 months.
- Iritis has been reported following treatment with both oral risedronate and intravenous pamidronate. This appears to be less of a problem in children than in adults but, in the event of eye problems developing, early referral to an ophthalmologist is advised.

Treatment with bisphosphonates should be undertaken only in centres where there is expertise in the use of these drugs.

Denosumab

Denosumab is a monoclonal antibody to RANKL that acts as an inhibitor. It has mainly been used in adults with hypercalcaemia of malignancy but there are some reports of its use in children with MAS [185], OI type VI [186] and hypercalcaemia of malignancy [187]. Like the bisphosphonates, it inhibits osteoclast activity but, unlike them, is not incorporated into bone. It is preferable to the bisphosphonates where renal glomerular function is reduced below 30%. It may cause delayed hypocalcaemia and rebound hypercalcaemia after cessation.

FGF23 Monoclonal Antibody (Burosumab [KRN23])

Burosumab is a recombinant human monoclonal antibody against FGF23. It has undergone phase 1-3 trials in growing children with X linked hypophosphataemic rickets (XLH). In 2018 burosumab became licensed by the FDA in the USA and available on the UK National Health Service to children with XLH who had growing skeletons above the age of 1 year. It is administered as a subcutaneous injection every two weeks and the doses are titrated primarily against fasting serum phosphate levels.

Although not currently licensed for other forms of phosphate wasting it theoretically would be effective in other forms of hypophosphatemic rickets associated with elevated FGF23 [188].

Corticosteroids

Corticosteroids, usually prednisolone, may be used in certain cases of hypercalcaemia, particularly those associated with excess vitamin D or its metabolites such as subcutaneous fat necrosis. They may also be a secondary cause of osteoporosis (e.g. DMD) and attempts to wean off the steroids should be made to try to improve this.

Loop diuretics

Furosemide is used in addition to rehydration as part of the treatment of symptomatic hypercalcaemia. It may also contribute to correction of severe hyperphosphataemia. It should be used with caution as it tends to increase urinary calcium excretion and may contribute to the development of nephrocalcinosis. Thiazides have the opposite effect.

Acetazolamide

Acetazolamide, a carbonic anhydrase inhibitor, has been reported to be of use as adjunct therapy in hyperphosphataemia.

Calcitonin

CT is occasionally used for its hypocalcaemic effects when severe hypercalcaemia has not responded to forced diuresis. Its use has now been largely supplanted by bisphosphonates.

Phosphate Supplements

Phosphate supplements form an important part of the treatment of hypophosphataemic conditions, especially hypophosphataemic rickets. Phosphate is rapidly absorbed from the GI tract and is also rapidly excreted, especially when TRP is low. Unfortunately, there are no slow-release preparations of phosphate available so it has to be given several times a day, preferably up to 4 or 5 times, in order to maintain adequate concentrations. It is often difficult to achieve this because of problems of compliance both because of the taste and frequency of administration. In addition, diarrhoea may limit the total dose.

Phosphate Binders

These are most useful in CKD when a reduced GFR limits phosphate excretion. Aluminium hydroxide has been largely supplanted initially by calcium carbonate but more recently by sevelamer (Renagel®). While calcium carbonate is effective at reducing plasma phosphate, it has been associated with the development of adynamic bone. Sevelamer is not absorbed by the gut and does not have the same problem. Sevelamer may also be useful in situations where soft tissue calcification arises as a result of hyperphosphataemia and has been reported to be of use in HFTC.

Cinacalcet (Mimpara®)

Cinacalcet acts by increasing the sensitivity of the parathyroids to plasma calcium. It shifts the CaSR/PTH curve to the left (Figure 10.2) and has been developed principally for the treatment of secondary hyperparathyroidism in CKD but there are also reports of its successful use in FBH [189, 190]. It is not clear if it is effective in homozygous forms of this condition. It is effective orally but requires twice daily administration.

Magnesium Supplements

Magnesium may be required in large quantities in hypomagnesaemic states, particularly where the hypomagnesaemia causes secondary hypoparathyroidism. Magnesium sulphate can be used but is likely to cause diarrhoea if used in large quantities. Magnesium glycerophosphate causes fewer such side effects. Magnesium sulphate can be given intramuscularly. A 50% solution contains 2 mmol/mL and the dose may be repeated as required to maintain concentrations above 0.7 mmol/L (1.6 mg/dL). It may also be given intravenously but must be given with caution as it causes intense vasodilatation if given too quickly.

Cathepsin K Inhibitor

Odanacatib promotes the degradation of type 1 collagen by inhibiting the action of cathepsin K, the enzyme responsible for removal of demineralized bone by osteoclasts. Recent trials have been halted because of an increased risk of stroke in adults [191].

Anti-Sclerostin Antibody

Unlike the bisphosphonates, denosumab or odanacatib, romosozumab acts on osteoblasts to promote the Wnt/β-catenin signalling pathway by inhibiting sclerostin, its antagonist. Its use in children has not been reported.

Recombinant Human Non-Specific Alkaline Phosphatase

Asfotase alfa® (Strensiq) is a bone-targeted form of NSALP that is proving successful in the treatment of hypophosphatasia [143]. It is given by subcutaneous injection twice weekly.

Palovarotene

Palovarotene is a selective RARγ agonist that not only has been developed for the treatment of smoking-related pulmonary emphysema but also inhibits heterotopic ossification and may prove useful in the treatment of fibrodysplasia ossificans progressiva [192]. Current trials are being undertaken in conditions such as fibrodysplasia ossificans progressiva and multiple osteochondromas.

Sodium Thiosulphate

Sodium thiosulphate has been reported to be of value in certain forms of soft tissue calcification such as HFTC [177] and the calcification associated with calciphylaxis of CKD [193]. It works by exchanging calcium phosphate for the much more soluble calcium thiosulphate. It can be administered orally, topically or by intravenous infusion and appears to be relatively safe although there are occasional reports of metabolic acidosis in association with its use.

Summary

This field can be broadly divided into problems with bone mineral metabolism and problems with bone structure and integrity. Although the two are closely linked and may impact each other, they are managed quite separately.

Disorders can both be acquired, such as vitamin D deficiency, or genetic, such as in OI. The medical history, the collection of appropriate samples during the time of dysregulation and a good understanding of the physiol-

Table 10.7 Biochemical changes observed in disorders associated with hypocalcaemia and hypercalcaemia.

	Corrected calcium	PTH	Phosphorus		Urine Ca : creat ratio	25(OH)D	1,25(OH)2D	
Subcutaneous fat necrosis	↑	↓	↑		↑	→	↑	
NSPHT	↑↑↑	↑↑↑	↓		↑↑	→↓	↑	
Williams syndrome	↑	↓	↑		↑	→	→↑	
Idiopathic infantile hypercalcemia	↑	↓	↑		↑	→↑	↑	
Familial hypocalciuric hypercalcemia	↑	→↑	→↓		↓	→	↑	
Primary hyperparathyroidism	↑	↑	↓		↓	→	↑	
Parathyroid tumours	↑↑	↑	↓		↑	→	↑	
Granulomatous disease	↑	↓	↑		↑	→	↑	
Immobilization	↑	↓	↑		↑	→	↓	
Vitamin D excess	↑	↓	↑		↑	↑	→↓	
	Corrected calcium	**PTH**	**Phosphorus**	**FGF23**	**Urine Ca:creat ratio**	**TmP/GFR**	**25(OH)D**	**1,25(OH)2D**
Autosomal dominant hypocalcaemia (ADH)	↓	→↓	↑	→	→↑	→	→	↓
Hypoparathyroidism	↓	↓	↑	→	↓	→	→	↓
22q deletion/DiGeorge syndrome	↓	↓	↑	→	↓	→	→	↓
Pseudohypoparathyroidism	↓	→↑	↑	→	↓	→	→	↓
Infant and adolescent non-rickets 'nutritional' vitamin D deficiency	↓↓	↑	↑	→	↓	→	↓↓	↓
'Nutritional' vitamin D deficiency with rickets	↓	↑	↓	↓	↓	→	↓↓	↓
Calcium deficiency	↓	↑	↓	↓	↓	→	→	↑
Vitamin D-dependent rickets type I (VDDRI, 1α hydroxylase deficiency)	↓	↑↑	↓	↓	↓	→	→	→↓
Vitamin D-dependent rickets type II (VDRRII)	↓	↑↑	↓	↓	↓	→	→	↑↑
Hypophosphataemic rickets (HPR)	→	→↑	↓	↑	→	↓	→	↓
Hereditary hypophosphataemic rickets with hypercalciuria (HHRH)	→	→	↓	↓	↑	↓	→	↑
McCune–Albright syndrome	→	→↑	↓	↑	→	↓	→	↓

ogy of bone and calcium metabolism is key to making a diagnosis and instigating appropriate treatment (see summary table of biochemical changes in hypo- and hypercalcaemia; Table 10.7).

Progress in the field of genetics over the last decade has opened the door to faster and more accurate diagnosis. It has also led to greater understanding of skeletal disorders and we are currently seeing the results of bench to bedside research. New therapies such as recombinant human non-specific alkaline phosphatase have dramatically altered prognosis for affected newborns with severe hypophosphatasia. For the first time in over two decades, pediatricians are starting to have alternatives aside from bisphosphonates in the treatment of childhood osteoporosis.

The next decade promises improved diagnosis, greater insight into underlying pathology and more treatment options for children with bone and mineral disorders.

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Weblinks

Bone mineral density
http://www.iscd.org/official-positions/2013-iscd-official-positions-pediatric/
Vitamin D and rickets
https://www.karger.com/Article/FullText/443136
22q deletion syndrome
https://maxappeal.org.uk
Williams syndrome
http://www.orpha.net/data/patho/Pro/en/WilliamsGuidelines_2010.pdf
Hypophosphatasia
http://www.sesepuvsq.fr/
X-linked dominant hypophosphataemic rickets
http://phex.mcgill.ca
Calcium-sensing receptor disorders
http://www.casrdb.mcgill.ca
Collagen variants in osteogenesis imperfecta and Ehlers–Danlos
http://www.le.ac.uk/genetics/collagen

11

Polyglandular Autoimmune Syndromes

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KEY LEARNING POINTS

- Failure of central immune regulatory mechanisms (notably the elimination of potentially self-reactive T cells) and peripheral regulatory mechanisms (such as the action of T regulatory cells) can give rise to autoimmune endocrine disease.
- The diagnosis of APS1 is challenging due to the variability of the early clinical picture, but early diagnosis is important to prevent serious complications and death.
- Increased awareness of the minor manifestations (prior to the development of any of the classic triad of mucocutaneous candidiasis, hypoparathyroidism and adrenal failure), together with measurement of anti-interferon antibodies and mutational analysis of the AIRE gene, will promote earlier detection.
- APS1 patients need regular and careful follow-up to prevent life-threatening situations; psychological support is also crucial.
- A number of patients who develop adrenal insufficiency already have an autoimmune disorder such as type 1 diabetes or autoimmune thyroid disease.
- In those with T1D, recurrent hypoglycaemia or unexplained hyperglycaemia should prompt consideration of the development of other associated autoimmune endocrinopathies such as adrenal failure and thyroid disease.
- Thyroid and gut dysfunction in early life are common manifestations of an expanding number of recognized immune dysregulation and deficiency syndromes; early-onset T1D is also commonly a feature.
- Growth failure is a common manifestation of many immune dysregulation and autoimmune endocrine disorders and tends to respond poorly to GH therapy.

Introduction

The autoimmune polyendocrinopathy syndromes (APS) are rare and cover a wide clinical spectrum of diseases with both monogenic and complex genetic aetiologies. The first manifestation is often in childhood or adolescence with an extremely variable presentation, which involves at least two endocrine glands being affected by autoimmune disease.

Patients have disturbed immune tolerance and their immune system fails to differentiate accurately between self and non-self. This may be due to an abnormality of thymic function or of peripheral tolerance or to a combination of both. The thymus normally eliminates T cells that have an autoreactive capability (negative selection) but, if this process is dysfunctional, autoreactive T cells may escape into the periphery. Tolerance of self also occurs in the periphery, such that responses by naive T cells against self-antigens that are not present in the thymus do not normally escalate into disease. Fundamental to this peripheral tolerance is a specific T-cell lineage, T regulatory cells (Tregs), which bear markers including $CD4^+$, $CD25^+$ and forkhead box protein P3 ($FoxP3^+$); Tregs suppress the immune responses of other cells.

Many immune diseases reflect an abnormality of both 'central' and 'peripheral' tolerance and have a complex genetic aetiology. The disorders APS1 (autoimmune polyendocrinopathy syndrome type 1) and IPEX (immune dysregulatory, polyendocrinopathy, enteropathy, X-linked) are key single gene disorders with a predominantly central and peripheral defect, respectively (Figure 11.1). From a conceptual point of view, it is easy to understand why a disorder associated with a tendency to target self may also fail to eradicate pathogens appropriately, leading to an increased susceptibility to infections in many of these conditions.

Brook's Clinical Pediatric Endocrinology, Seventh Edition. Edited by Mehul T. Dattani and Charles G. D. Brook.

Companion website: www.wiley.com/go/dattani/brookcpe7

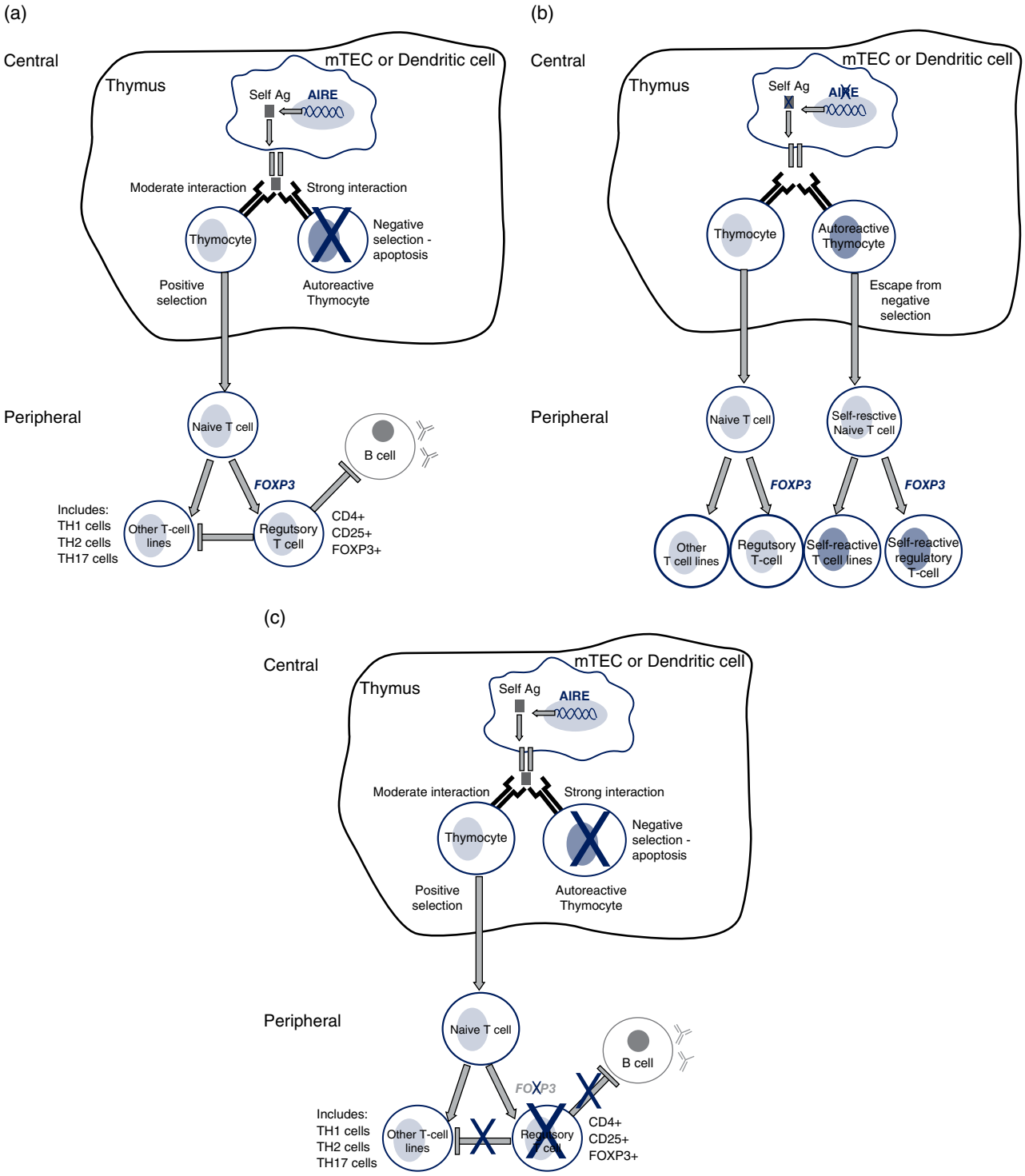

Figure 11.1 It is still not fully clear how AIRE or FOXP3 deficiency leads to autoimmunity, but the current model is that AIRE plays a predominant role in the development of central immune tolerance, whereas FOXP3 is predominantly linked with the development of T-regulatory cells and peripheral immune tolerance. (a) In the current theory, self-antigens are presented by thymic epithelial cells (mTECs) and dendritic cells within the thymus to developing thymocytes. Those that show a strong degree of reactivity to the self-antigens are negatively selected out and removed by apoptosis. Those that show a moderate degree of reactivity to self-antigen are positively selected and pass to the periphery to develop further into different T-cell lineages, including T-regulatory cells. The development of the latter is related to the cells' expression of FOXP3, and regulatory T cells are essential in controlling peripheral immunity. (b) In APS1 the AIRE gene is deficient and so this downregulates the expression of self-antigens in the thymus so these are not presented to the developing thymocytes. Self-reactive thymocytes therefore escape negative selection and apoptosis, and are exported to the periphery together with non-self reactive thymocytes. Both groups of naïve T cells can then develop into a range of T-cell subtypes, leaving self-reactive mature T cells in the periphery to cause autoimmunity. (c) In those who are FOXP3 deficient, the usual process of positive and negative selection occurs in the thymus; however on reaching the periphery the lack of FOXP3 expression in the naïve T cell leads to a deficiency of the development of regulatory T cells and therefore a peripheral immune system that is not kept in check and is over-reactive.

Autoimmune Polyendocrinopathy Syndrome Type 1 (APS1)

Definition

APS1, also known as the autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy syndrome (APECED), is a rare and frequently debilitating disorder of childhood and adolescence. It is inherited as an autosomal recessive condition; heterozygotes typically have no manifestations, although the occasional individual with a single monoallelic dominant negative mutation has recently been reported, developing a less severe form of APS1 at a later age and with better outcomes [1]. The female–male ratio is close to 1. The clinical diagnosis of APS1 classically requires the presence of two of the three cardinal components: chronic mucocutaneous candidiasis (CMC), autoimmune hypoparathyroidism and autoimmune adrenal failure [2, 3]. Only one of these is required if a sibling has the syndrome or if there are also two minor manifestations present [2], which include endocrine and non-endocrine manifestations. Clinicians need to remember that just about all tissues can be affected in patients with APS1.

Large cohorts of patients have been reported from several countries, including Finland [3], Norway [4, 5], Israel [6], Sardinia [7], Northern Italy [8] and Northern America [2, 9]. Although a rare disorder in most countries (about 2 or 3 cases per million in the UK), it shows a founder effect leading to a much higher prevalence in certain populations: Finns 1:25,000 [3], Iranian Jews 1:9,000 [6] and Sardinians 1:14,500 [7]. There are also differences in phenotypes between different populations: for example, CMC and adrenal failure are among the commonest manifestations in most patients of European descent but present in only about 20% of Iranian Jews.

Clinical Features and Course

The first manifestation is typically mucocutaneous candidiasis, which develops in infancy or early childhood. Hypoparathyroidism characteristically develops around the age of 5–7 years and adrenocortical failure by the age of 13 years (Figure 11.2) [8, 10]. The complete evolution of the three cardinal features usually occurs in the first 25 years, with additional minor manifestations continuing to appear until at least the fifth decade. Although this temporal sequence of appearance of the major manifestations is often observed in childhood, APS1 subjects frequently present in other ways, either with one cardinal feature and several minor manifestations or with several minor manifestations and characteristic ectodermal dystrophy. The variability in the early clinical picture can make the diagnosis of APS1 challenging and patients may come to medical attention because of signs such as urticarial rashes, recurrent fever, abdominal distention and failure to thrive.

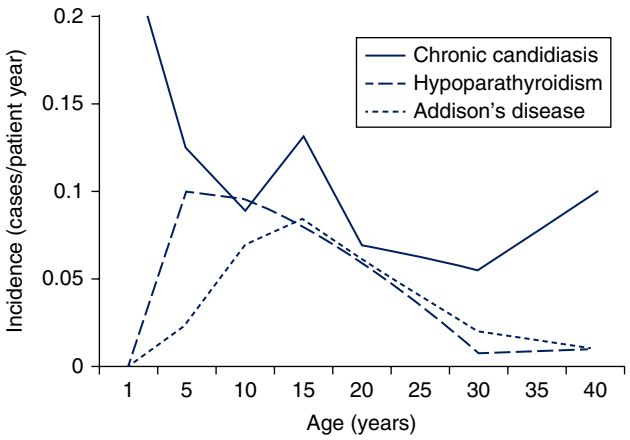

Figure 11.2 Incidence of the three most common components of APS1 according to age.

In European populations, the median number of disease components in any individual patient throughout their life is 5, with up to 10 manifestations in some subjects [5]. The cardinal triad occurs in around 60% of subjects but there may be substantial delay in diagnosis in the early years when the rarer components, particularly hepatitis, periodic rashes with fever, keratoconjunctivitis, chronic diarrhoea or vitiligo, may dominate the clinical picture.

A recent American case series found a similar number of APS1 patients affected with endocrinopathies but a much higher predominance of non-endocrine manifestations such as urticarial eruptions (66%), hepatitis (42.9%) and intestinal dysfunction (80%) [9], which were also some of the earliest in this patient group. Two other significant manifestations in the group were Sjögren-like syndrome (42.9%) and pneumonitis (40%).

Patients who present initially with adrenal insufficiency rather than with candidiasis tend to develop fewer components than others [3, 11]. It has also been reported that the earlier the first component presents, the more likely it is that multiple components will develop [2, 8]. Table 11.1 lists the cardinal and more common minor manifestations and their reported frequencies.

Cardinal Manifestations

Chronic Mucocutaneous Candidiasis (CMC)

Chronic or periodic mucocutaneous candidiasis is commonly the first manifestation of the syndrome, occurring as early as 1 month of age but more typically in the first 2 years of life, and it should alert the clinician to the possibility of APS1. It is frequently mild or

Table 11.1 Frequencies of the major and main minor components of APS1.

Disease	Frequency (%)
Main manifestations	
Chronic mucocutaneous candidiasis	72–100
Autoimmune hypoparathyroidism	73–93
Autoimmune adrenal failure	63–100
Common minor manifestations	
Autoimmune endocrinopathies	
Hypergonadotropic hypogonadism	17–69
Autoimmune thyroid disease	4–31
Type 1 diabetes mellitus	0–33
Pituitary defects	7–17
Gastrointestinal components	
Atrophic gastritis ± pernicious anaemia	13–49
Malabsorption	10–80
Cholelithiasis	44
Chronic active hepatitis	4–43
Skin autoimmune diseases	
Vitiligo	8–37
Alopecia	17–40
Urticarial-like erythema with fever	15–66
Ectodermal dysplasia	
Nail dystrophy	10–52
Dental enamel hypoplasia	40–86
Tympanic membrane calcification	33
Other manifestations	
Keratoconjunctivitis	2–35
Hypo/asplenia	9–40

Data from European and North American patients [2–5, 8–12]. Iranian Jews have distinctly different frequencies from the other populations and have been excluded.

intermittent and mostly responds well to periodic systemic anti-candidal treatment, although some cases can be severe with chronic inflammation and the development of hyperkeratotic plaques. CMC does not develop in some subjects until adulthood [2, 3] but it is the most frequently occurring cardinal manifestation, present in 73–100% of patients. Oral candidiasis is commonest but esophagitis is also found in around 20%, causing substernal pain and odynophagia. Infection of the intestinal mucosa leads to abdominal discomfort and diarrhoea. Candidal infection can also affect the vaginal mucosa, nails and skin. Chronic untreated candidiasis can lead to squamous cell carcinoma and therefore needs treating aggressively; regular dental follow-up is essential.

CMC in APS1 is a manifestation of autoimmunity to endogenous cytokines [13]. Interleukins normally produced by Th cells (IL-17A, IL-17F and IL-22) induce antimicrobial peptides and chemokines with direct antifungal activity and chemoattractants that recruit neutrophils in response to invading fungi. In APS1, neutralizing autoantibodies to these T-helper cell cytokines develop, impairing the body's response to candidal infections.

Virtually all patients with APS 1 develop autoantibodies to interferons, particularly IFN-α and IFN-ω, at a stage before the onset of symptoms. Their role in the susceptibility to CMC infection is unclear [14].

Hypoparathyroidism

This is frequently the first endocrine feature of APS1, with a peak incidence between 2 and 11 years of age. Hypoparathyroidism occurs in around 75–95%, although there appears to be a slightly reduced penetrance and later age of onset in males [15]. Hypoparathyroidism is often asymptomatic but may present with tetany, paraesthesia, diarrhoea and seizures. Presentation may be precipitated by fasting, a low calcium diet or high phosphate intake.

The diagnosis is confirmed by a low or undetectable plasma parathyroid hormone (PTH) concentration in the presence of hypocalcaemia. Hyperphosphataemia and hypomagnesaemia are common, with low urinary calcium excretion. Autopsy studies of parathyroid glands from these patients show atrophy and an infiltration with mononuclear cells. APS1 should be considered in every case of primary hypoparathyroidism in childhood.

Adrenal Failure

Autoimmune adrenal failure (Addison's disease) is typically the third of the cardinal manifestations to present in APS1, with a peak incidence around 13 years [2–4, 6, 8, 10, 11]. In most populations of APS1 patients, it occurs less frequently than the other major components (72–100%). Destruction of the adrenal cortex may develop gradually and deficiencies of cortisol and aldosterone can appear in either order up to 20 years apart [11].

Adrenal failure is a life-threatening condition with symptoms of fatigue, weight loss, increased pigmentation of the skin, hypotension, salt craving and abdominal pain. At autopsy, the adrenals of these patients are atrophic, with the adrenal cortex being almost completely destroyed and having an extensive inflammatory cell infiltrate. Diagnosis of adrenal insufficiency is confirmed by a normal or low cortisol concentration with increased adrenocorticotropic hormone (ACTH) and a subnormal cortisol response to synthetic ACTH stimulation.

A temporary hypermineralocorticoid-like state is seen in some patients with cortisol deficiency, paradoxically

leading to hypokalaemia [11, 12]. Deficiency of aldosterone may be heralded by postural hypotension or salt craving and is confirmed by a raised plasma renin activity even before the development of overt electrolyte disturbance.

Minor Manifestations

Autoimmune Endocrinopathies

Primary hypogonadism is the commonest minor manifestation of APS1 in females, occurring in 17–61% of cases. It is almost invariably accompanied by adrenal failure and is 3 times more common in females compared with males, as it is thought that the blood–testis barrier may protect the Leydig cells from autoimmune attack. About half of APS1 females with hypogonadism present with primary amenorrhoea or arrested puberty and the remainder develop secondary amenorrhoea and premature menopause. Male hypogonadism has been reported from puberty onwards. One male patient has been reported with azoospermia and possible antisperm autoimmunity [11]. Diagnosis is made by elevated LH and FSH concentrations with low oestrogen or testosterone concentrations.

Type 1 diabetes mellitus (T1D) is relatively infrequent in APS1 compared with other polyendocrinopathy syndromes. There is an age-related penetrance with a peak presentation towards middle age [12]. There is a wide range in the reported prevalence between different APS1 populations from 0 to 23%, often depending on age of the cohort.

Autoimmune thyroid diseases (Hashimoto's thyroiditis or primary atrophic thyroiditis) occur in up to 20% of cases. The age of presentation varies from around 10 years for Hashimoto's thyroiditis to 17 years for primary atrophic thyroiditis [2, 3, 8]. Hyperthyroidism is very rare.

Pituitary defects such as lymphocytic hypophysitis or autoimmune pituitary disease have occasionally been described (<5%) and can lead to single or multiple hormonal defects [10]. Cases of secondary hypogonadism [3], growth hormone deficiency [12] and idiopathic diabetes insipidus [8] have all been reported; a recent case series in the USA reported growth hormone deficiency in 17.1% of an APS1 cohort [9].

Gastrointestinal Components

Although a minor component of APS1, gastrointestinal manifestations commonly cause significant morbidity in children but are often overlooked with other problems taking priority. Symptoms and pathogenesis are poorly understood and can often occur simultaneously with other disorders that may show similar symptoms, thereby masking the problem.

Chronic atrophic gastritis, an organ-specific autoimmune disease affecting gastric parietal cells and intrinsic factor, affects up to a third of patients with APS1, with a peak incidence at 10–20 years [2–4, 6, 8, 10, 11]. It can lead to a megaloblastic anaemia due to vitamin B_{12} deficiency (pernicious anaemia), usually in early adulthood, or a microcytic anaemia because of iron deficiency. B_{12} deficiency can also lead to a peripheral neuropathy, spinal cord degeneration and personality change.

Malabsorption is thought to be due to a variety of causes including villous atrophy, exocrine pancreatic insufficiency, intestinal infections (*Giardia lamblia*, *Candida* or *Clostridium difficile*), defective bile acid reabsorption and intestinal lymphangiectasia [8, 11, 16]. Autoimmune destruction of the enterochromaffin cells of the small intestine leading to deficiency of cholecystokinin and serotonin has also been implicated, as has autoimmunity to enteric defensins, leading to loss of Paneth cells [17]. The malabsorption typically presents with periodic or chronic diarrhoea, usually with steatorrhoea, but it may be associated with constipation. It can lead to weight loss, growth retardation and erratic absorption of medications.

It can be a characteristic feature of an early atypical presentation of APS1 in the first year of life, being an initial manifestation in around 10% of patients. Chronic diarrhoea is the first manifestation in the majority, with severe constipation in around 2%; the majority of patients with APS1 have periodic intestinal dysfunction [18]. There is a strong association with the hypocalcaemia of hypoparathyroidism because hypocalcaemia impairs the secretion of cholecystokinin leading to a failure of normal gall bladder contraction and pancreatic enzyme secretion that leads to steatorrhoea. Severe malabsorption can lead to the development of hypocalcaemia without hypoparathyroidism; in this situation patients also have a low phosphate and a high PTH concentration.

Cholelithiasis is present in up to 40% on ultrasonography [8], is frequently asymptomatic and is thought to be secondary to disruption of the enterohepatic circulation.

Chronic active hepatitis develops in 5–30% of cases [2–4, 6, 8, 10–12, 18]. The clinical course varies from chronic but asymptomatic in most cases to the development of cirrhosis or fulminant hepatic failure with a potentially fatal outcome. It may present in early childhood and be the first manifestation of APS1. The risk of hepatitis is low after adolescence. Elevation of serum alanine aminotransferase for more than 3 months when no other cause such as viral- or drug-induced hepatitis can be found is an indication for liver biopsy [3]. Clinicians should be particularly vigilant in the early weeks after the identification of abnormal liver function in APS1 subjects because rapid decompensation to frank liver failure may occur.

Coeliac disease is recognized in APS1 and should be screened for but it is not a common association.

Skin Autoimmune Diseases

Vitiligo can appear at any age but most commonly in childhood, affecting up to a quarter of APS1 patients [2–4, 6, 8, 10, 11]. It is highly variable in extent and often worsens with time.

Alopecia affects about a third of patients and can involve all body sites in varying degrees [2–4, 6, 8, 10, 11, 18]. It can develop rapidly and at any age.

Recurrent urticaria with fever has been reported as an unusual manifestation in about 10% of patients during childhood but a case series from the USA found an enrichment of non-endocrine APS1 manifestations in its cohort and urticaria was found in two-thirds of patients [9]. It may persist for many years and is strongly associated with uveitis. High concentrations of immunoglobulin G (IgG) and circulating immune complexes are found and skin biopsy reveals a lymphoplasmacytic vasculitis [11].

Other Manifestations

Ectodermal dystrophy affects the nails and tooth enamel (Figure 11.3). The pitted nails are unrelated to candidal infection and can be an important clue to the diagnosis of APS1 but they can be difficult to distinguish from fungal infections. Dental enamel hypoplasia has been reported in 40–75% of patients [2–4, 6, 8, 10, 11, 18]; originally this was thought to involve only permanent teeth but hypoplastic changes have also been reported in deciduous teeth. Enamel hypoplasia can precede hypoparathyroidism and is unrelated to serum calcium concentrations. Even in the absence of ear infection, a third of patients have calcified plaques on their tympanic membranes [3, 11]. Ectodermal dystrophy is thought to be autoimmune in origin although the antibody target has not been identified.

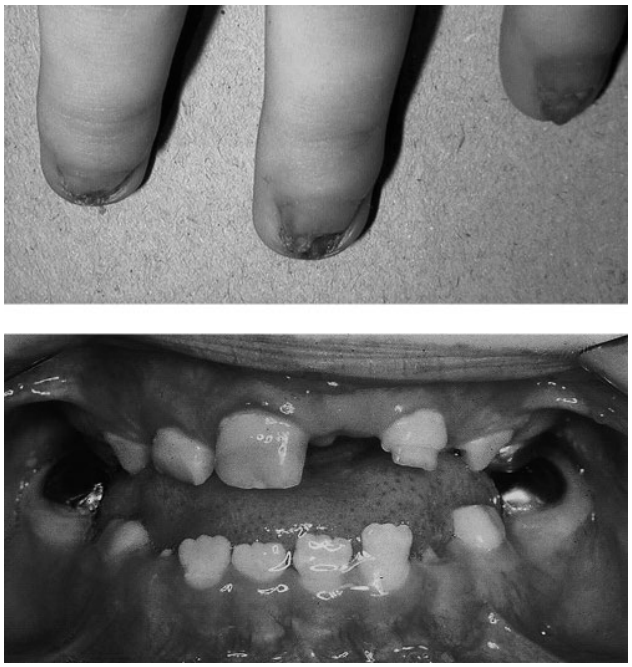

Figure 11.3 Ectodermal features of APS1 illustrating the nail dystrophy and the dental enamel hypoplasia.

Keratoconjunctivitis incidence varies from 10 to 40% [2–4, 6, 8, 10, 11]. It can be the first manifestation of APS1. The initial symptoms are intense photophobia, blepharospasm and lacrimation; progressive corneal scarring, permanent visual impairment and even blindness are not infrequent. Some patients enter a quiescent phase around 10 years after onset. The severity of the eye findings has no correlation to that of the systemic manifestations of APS1.

Autoimmune retinopathy leading to photoreceptor degeneration and visual loss has been described as part of the APS1 phenotype [19].

Acquired lipodystrophy is known to be associated with autoimmune disorders and has recently been reported in a child with APS1 [20] in whom progressive generalized lipodystrophy presented before the onset of any of the other manifestations, including candidiasis.

Asplenia or hyposplenism has been documented by ultrasonography or suggested by haematological parameters in up to 15% of APS1 cases. It may be congenital or acquired secondary to progressive autoimmune-mediated destruction or vascular insult to the spleen. It is suggested by a blood smear including Howell–Jolly bodies and thrombocytosis. It causes an additional secondary immunodeficiency, rendering subjects susceptible to pneumococcal sepsis.

Renal involvement has been reported in 15–20% of cases, leading to moderate to severe renal failure, the majority being due to tubulo-interstitial nephritis. In many cases treatment with immunosuppression, haemodialysis or renal transplantation is required [21]. Circulating antibodies against the distal part of the nephron are a frequent association in these patients, although the target antigen has not been identified and their significance is uncertain. Other renal problems in APS1 patients include iatrogenic nephrocalcinosis and renal tubular acidosis type 1 of which the only sign may be impaired renal function with normal urinalysis, kidney stones and hypertension [21].

An increase in severe and atypical *infections* has been shown in APS1 patients [22], which would not be surprising given that these patients have antibodies

to type 1 interferons and Th17-related cytokines. Infectious complications need more attention and additional surveillance in this patient group.

Pulmonary disease including primary pulmonary hypertension, autoimmune bronchiolitis and bronchiectasis with pneumonitis, a relatively common manifestation, has been seen sporadically in some cohorts [9]. A potential autoantigen, the potassium channel-regulating protein, has been identified [23].

Rarer associations: several cases of selective IgA deficiency and hypergammaglobulinemia have been reported [12]. Many patients have tuberculin anergy but whether this indicates an abnormal susceptibility to tuberculosis is unclear. Neoplasia, most commonly squamous carcinoma of the oral mucosa (particularly in subjects with chronic oral *Candida* who smoke) and adenocarcinoma of the stomach, is also seen. Other rare manifestations are listed in Table 11.2.

Sudden death is well recognized in APS1 patients and their siblings and from post-mortem studies of subjects in whom the diagnosis was not suspected. It is presumed that deaths are due to undiagnosed adrenal failure, fulminant sepsis, hypoparathyroidism or a combination of these pathologies.

Table 11.2 Rarer minor manifestations having reported association with APS1 [1–6, 9, 13].

Rare components of APS1
Immunological
Selective IgA deficiency
Hypergammaglobulinemia
Tuberculin anergy
Increased infection risk
Renal
Tubulo-interstitial nephritis
Iatrogenic nephrocalcinosis
Renal tubular acidosis
Hypertension
Kidney stones
Neurological
Intracranial calcification
Progressive myopathy
Connective tissue
Sjögren syndrome
Cutaneous vasculitis
Scleroderma
Rheumatoid arthritis
Lupus-like panniculitis
Haematological
Pure red cell aplasia
Autoimmune haemolytic anaemia
Malignant
Oral squamous cell carcinoma
Oesophageal carcinoma
Adenocarcinoma of the stomach
Ophthalmic
Iridiocyclitis
Optic nerve atrophy
Autoimmune retinopathy
Cataracts
Respiratory
Primary pulmonary hypertension
Autoimmune bronchiolitis
Bronchiectasis
Others
Metaphyseal dysplasia
Acquired lipodystrophy
Lymphocytic myocarditis
Coeliac disease

Genetics

The gene defective in APS1 is located on chromosome 21q22.3. It is named the **a**uto**i**mmune **re**gulator or *AIRE* gene that encodes a nuclear protein containing several domains involved in nuclear transport, DNA binding, homomultimerization and transcriptional activity, including two zinc fingers. Immunostaining reveals that it is concentrated in nuclear 'speckles' expressed in a variety of tissues of the immune system but predominantly in the medullary thymic epithelial antigen-presenting cells, where it is thought to play an important role in the central induction of self-tolerance. Medullary thymic epithelial cells express a wide array of tissue-specific antigens from different organs in the body via MHC class II, which is important to eliminate autoreactive T cells, a critical step in inducing immune tolerance to self and preventing autoimmunity. The AIRE protein regulates the expression of these self-antigens in the medullary thymic epithelial cells and so plays a key role in this process. In subjects with *AIRE* mutations, reduced expression of tissue-specific antigens in the thymus means that autoreactive T cells escape to the periphery leading to autoimmunity in selected organs.

AIRE is also expressed in peripheral dendritic cells and so may play an additional role in the maintenance of peripheral immune tolerance, although this has

yet to be clarified. A markedly reduced number of CD4+CD25+ Tregs have been found in the peripheral blood of APS1 subjects, which could be a secondary effect of *AIRE* mutations [24].

Antibodies to cytokines are an early feature of APS1, which points to an early, focused abnormality of immune function [25]. Due to this and the fact that clinical manifestations can occur very early in APS1, it has been realized that the naïve autoreactive cells must become activated soon after birth, so there must be more underlying the pathogenesis. It has been hypothesized that defective AIRE function affects the thymic microenvironment so that the T cells exported are preactivated and the autoantibody-producing B cells are already primed [22].

Over 100 different disease-causing mutations have been described in the *AIRE* gene. These include point mutations, insertion and deletions and are spread through the whole coding region of the gene. Mutations affecting splice sites have also been reported, as have large genomic deletions of the AIRE gene. The most frequent *AIRE* mutations include the founder Finnish mutation in exon 6 (p.R257X) and the common northern European mutation in exon 8 (964del13). This 13-bp deletion is seen frequently in Norwegian patients and in whites from the USA and UK, where it accounts for more than 70% of all mutant *AIRE* alleles. Many patients with this 13-bp deletion carry the same haplotype over the 21q.22 region, which is evidence for a founder effect. The common Finnish *AIRE* mutation is also fairly prevalent (5–30%) in other subjects of white European ancestry. Additional common mutations are found in isolated populations, such as a mutation in exon 3 (p.R139X) found in Sardinians and a mutation in exon 2 (p.Y85C) in the Iranian Jewish population. One case series from the USA found a higher incidence of APS1 patients with either just one or no identifiable AIRE gene mutation (16.8% of cases) and most of the remainder were compound heterozygotes [9]. This population therefore appears more genetically diverse than the previously well-described European patients.

Most of these mutations are believed to form null alleles that lead either to the synthesis of a truncated product or to the production of a nonsense transcript with a rapidly degraded mRNA. Missense mutations in the amino terminus of the protein may inhibit function by preventing multimerization or altering the distribution of the AIRE protein.

In several instances, only one mutant allele of the *AIRE* gene has been reported in APS1 patients, suggesting that the second mutation might be located in the regulatory regions of the gene. In 2001 however, Cetani et al. [26] found a mutation (G228W) in an Italian kindred that appeared to act in a dominant fashion. More recently multiple cases and families have been identified who have APS1-like features together with heterozygous mutations in the PHD1 domain of the AIRE gene [1]. It appears that these mutations are exerting a dominant negative effect and affected patients have a narrower organ-specific phenotype, later onset of symptoms, better long-term outcomes and incomplete penetrance. Interferon autoantibodies also appear to be less prevalent in this patient group, which has led to the hypothesis that mutations in AIRE may be more widespread in patients with autoimmunity than previously thought and that some isolated cases of autoimmune disease, such as vitiligo and pernicious anaemia, may have a single, rare, dominant negative variant of the AIRE gene. The AIRE protein multimerizes to form AIRE tetramers, which are essential for normal AIRE function. PHD1 mutations interfere with this multimerization and therefore can have an effect on AIRE function, even in the presence of a 'normal' gene copy, i.e. the mutant allele is suppressing the wild-type allele in a dominant negative manner.

There is still much that is not understood regarding the genetics of APS1, including the early onset of autoimmunity, the variation in phenotype in patients with the same genotype and why CMC, hypoparathyroidism and adrenal failure are the most common features. There is a correlation between the underlying *AIRE* mutation and disease components – candida infections are less common in those homozygous for the 964del13 compared to patients with p.R257X or p.R139X mutations and they are exceedingly rare among Iranian Jews with the p.Y85C mutation, as is adrenal failure. The 964del13 mutation is associated with a higher incidence of alopecia and the p.G228W mutation with autoimmune thyroiditis [22]. This may be related to the AIRE protein being truncated at different points, leading to different effects on gene function, or certain mutations may leave some residual activity or allow some pure wild-type tetramers to form, leading to a milder phenotype. A recent study has found that those with splicing site mutations have a milder phenotype with later disease onset compared with other mutation types [5].

It is possible that the specific manifestations that develop in a particular APS1 patient may depend on alleles at other loci such as human leucocyte antigens (*HLA*), because the same *AIRE* mutations are associated with varying phenotypes and clinical course, even among affected siblings [25]. No strong associations between APS1 manifestations and *HLA* alleles have been found but *HLA-A28* shows a weak association with hypoparathyroidism, keratopathy and alopecia in this disease and *HLA-A3* with ovarian failure. Addison's disease has been associated with *HLA DRB1*03* and alopecia with *HLA DRB1*04*, *DQB1*0302*. T1D shows a negative correlation

with *DRB1*15*, *DQB1*060*. Thus, *HLA* polymorphisms may explain some of the variability in phenotype seen in APS1, although no association between HLA type and autoantibodies is seen in APS1 patients [27]. No correlation between cytotoxic T lymphocyte antigen 4 (*CTLA4*) gene polymorphisms and APS1 has been found to date [10] but a negative correlation has been shown between the insulin gene polymorphism and the development of T1D in these subjects [28]. The factors determining an individual phenotype are not understood and it is likely that there are several loci involved.

Autoantibodies and Pathogenesis

The pathogenesis of many of the manifestations of APS1 is unclear but autoimmunity appears to be involved in the development of most of the major and minor manifestations. The autoimmune process is presumed to be T cell mediated but these individuals develop a variety of autoantibodies against proteins in the affected organs, frequently against intracellular enzymes involved in hormone or neurotransmitter biosynthesis. It is thought that these autoantibodies are not pathogenic *per se* but are a sign of ongoing T-cell reactivity and are a valuable disease marker.

One recently identified group of autoantibodies is directed against interferons, particularly IFN-α and IFN-ω, and has almost 100% prevalence in APS1 subjects, regardless of the clinical picture, mutation type, disease duration, gender or ethnic background [9, 14]. Anti-interferon autoantibodies have been found to be present at a very early stage in the first year of life before the onset of symptoms [29] and persist, being present after over 30 years of disease. They have not been found in any subjects with isolated AAD or APS2 or in unaffected heterozygotes, so they appear to be disease specific to APS1. This clearly provides an excellent tool to aid in the diagnosis of APS1 in the prodromal stage or in atypical cases as the sensitivity, specificity and predictive value of these anti-interferon autoantibodies is >98%. It also raises the intriguing possibility that these autoantibodies may modulate the expression of the immune response directly. The early onset of these antibodies suggests that the autoimmune process begins soon after birth or even earlier.

Another group of antibodies in this patient group are those to the interleukins, IL-17A, IL-17F and IL-22. These are found in 90% of APS1 cases and correlate clinically with the presence of CMC. IL-17 and IL-22 induce production of chemokines and antimicrobial peptides with direct antifungal activity; IL-22 also promotes epithelial barrier integrity. These antibodies are undetectable or at low concentrations in healthy siblings or unaffected relatives.

Steroid 21-hydroxylase (P450c21) and cholesterol side-chain cleavage enzyme (P450scc) are the major adrenal autoantigens; P450scc is also the major gonadal autoantigen in APS1 patients [30]. Antibodies against at least one of P450scc, steroid 17β-hydroxylase (P450c17) and P450c21 were found in over 90% of APS1 patients with and in 21% of those without adrenal failure. The presence of antibodies for at least one of these three enzymes correlates significantly with gonadal failure in female but not male patients [4, 8]. This is possibly due to the blood–testis barrier protecting the Leydig cells from immunological attack.

Adrenal cell autoantibodies are frequently detectable in patients with candidiasis or hypoparathyroidism without adrenal failure. These patients are almost certain to develop adrenal failure, although the antibodies may precede clinical AAD by more than 18 years [29]. In male APS1 hypogonadal patients, TSGA10 appears to be a potential autoimmune target but it does not appear to correlate with clinical symptoms so its relevance is uncertain. Several other novel autoantigens have been identified by proteome arrays, including the prostate-specific enzyme transglutaminase 4 (TGM4) that has been found to have an association with male infertility [31]. Also identified are the protein disulphide isomerase-like testis expressed (PDILT) and melanoma antigen B2 (MAGEB2), which are both expressed in testicular germ cells; autoantibodies are found in both males and females with APS1, but no clear clinical significance of these autoantibodies has been identified [32].

Autoantibodies to the extracellular domain of the calcium-sensing receptor (CaSR) have been reported in idiopathic hypoparathyroidism and in up to 86% of subjects with APS1 [33, 34]. This has not been replicated in all studies, probably because of differences in assay technique and sensitivity. In a few cases CaSR antibodies have stimulated the receptor, leading to inhibition of PTH secretion and hypocalcaemia, which is an alternative explanation for hypoparathyroidism other than just gland destruction. In addition, antibodies against a novel parathyroid-specific antigen, NALP5 (NACHT leucine-rich repeat protein 5), have been found in about half of APS1 patients with hypoparathyroidism [5, 35]. The prognostic significance and pathophysiological role of these autoantibodies remains undetermined.

Glutamic acid decarboxylase (GAD)-65 autoantibodies have been found in 75% of APS1 patients with diabetes, sometimes over 30 years before the onset of symptoms [29] but these are non-specific and not independently associated with diabetes in APS1 unlike in isolated T1D; they are also found in 40% of non-diabetic APS1 patients. Antibodies against the IA-2 tyrosine phosphatase-like protein and insulin are less common in these patients compared with non-APS1 T1D but have a

higher specificity (96–100%) when they do occur. As in isolated T1D, a combination of autoantibodies in APS1 has been suggested to provide a higher predictive value than the presence of a single, isolated diabetes autoantibody. Circulating antithyroid antibodies have been found to be a poor marker for predicting hypothyroidism in APS1 [8].

The main autoantigens for hepatitis in APS1 appear to be cytochrome P450 1A2 (CYP1A2), P450 2A6 (CYP2A6) and aromatic L-amino acid decarboxylase (AADC) [36]. CYP1A2 appears particularly to be a highly specific but insensitive marker for APS1 hepatitis. Liver–kidney microsomal (LKM) autoantibodies have been found in 50% of APS1 patients with chronic active hepatitis and in 11% of APS1 patients without increased concentrations of hepatic enzymes [36]. Other hepatic autoantigens associated with non-APS1 autoimmune hepatitis, such as smooth muscle and antinuclear antibodies, are not found. Tryptophan hydroxylase (TPH) autoantibodies have also been found to be a sensitive predictor of autoimmune hepatitis in APS1 [30]. Although a rise in antibody titres to liver antigens may predate biochemical evidence of liver disease, raised autoantibodies are not found in all APS1 patients with autoimmune hepatitis at biopsy. This, together with the broad spectrum of autoantigens found, suggests heterogeneity in pathogenesis as well as outcome.

Anti-parietal cell and intrinsic factor autoantibodies precede parietal cell atrophy. Villous atrophy is associated with endomysial and/or tissue transglutaminase (TTG) autoantibodies [8]. Gastrointestinal dysfunction has been associated with autoantibodies to TPH (48% cases), histidine decarboxylase (HDC) and GAD-65 [30]. TPH and HDC are associated with the destruction of serotonin-producing enterochromaffin and endochromaffin cells, respectively.

Vitiligo in APS1 is associated with the presence of complement-fixing melanocyte autoantibodies and has been associated with antibodies to the transcription factors SOX9 and SOX10 [37] and to AADC [30]. Antibodies to tyrosine hydroxylase are found in APS1 subjects with alopecia areata [10].

30% of APS1 patients have antibodies to the tubular basement membrane in the distal part of the nephron but this seems to have poor correlation with the development of tubulo-interstitial nephritis and renal disease, so their relevance is unclear [21].

Other antibodies that have been described but have unclear clinical significance include KCNRG, a putative potassium channel regulator identified as a pulmonary self-antigen [23], and tudor domain containing 6 (TDRD6), a pituitary protein potentially associated with clinical hypopituitarism or hypophysitis [38].

Measurement of autoantibodies may be of limited use in patients with APS1 in determining their risk of developing new components, because the sensitivity of the antibody test may frequently be less than the patient's pre-existing risk of the complication. They can however help give some prediction of future disease manifestations but fluctuating concentrations have been reported in APS1 for several antibodies including 21OH, tyrosine hydroxylase, SCC, IL-22 and GAD65 [5].

There are certain autoantibodies that are almost exclusive to APS1, including AADC, CYP1A2, tyrosine hydroxylase, TPH, IFN-α and IFN-ω. This unique spectrum of autoantibodies can thus help to differentiate APS1 from other autoimmune diseases [14, 30] (Table 11.3).

Diagnosis of APS1

Perheentupa found the classic criteria (two out of three cardinal manifestations) to be fulfilled in 22% cases in 5 years, in 67% in 10 years, in 89% in 20 years and in 93.5% in 30 years [11]. The classic triad may take many years to evolve and the diagnosis is often missed. To make a prompt diagnosis, suspicion should be high in patients under 30 years with mucocutaneous candidiasis, hypoparathyroidism, primary adrenal failure, ectodermal dystrophy, keratoconjunctivitis, prolonged diarrhoea, vitiligo or autoimmune hepatitis. Such patients should be checked for other manifestations, particularly the oral or ophthalmic components and the sometimes subtle nail signs of ectodermal dystrophy.

A review of American APS1 patients found that urticarial eruption, intestinal dysfunction and enamel hypoplasia commonly developed before the presentation of the classic criteria. In fact, if these were added to the classical diagnostic triad, diagnosis would have occurred ~4 years earlier and would have had implications for preventing life-threatening presentations before diagnosis [9]. This highlights the need to be vigilant for all manifestations, 'major' or 'minor', associated with APS1 and the threshold should be low for initiating genetic and autoantibody screening. The problem is that patients often present to dermatologists, gastroenterologists, dentists or ophthalmologists early in their disease before manifesting the classic endocrine conditions.

Diagnosis is also delayed not only because many children present with several 'minor' manifestations rather than one of the three 'major' components but also because of the long interval between the development of the first and second manifestation. Up to two-thirds of patients are not diagnosed until admission to hospital with acute adrenal insufficiency or hypocalcaemic crisis: nearly half of these already have one major component of APS1 present [4]. Increased awareness of APS1 is

Table 11.3 The identified autoantigens in APS1 for the commoner disease components.

Disease component	Autoantigens
APS1 generally (non-tissue specific)	IFN-α[a], IFN-ω[a]
Major manifestations	
Chronic mucocutaneous candidiasis	IL-17A[a], IL-17F[a], IL-22[a]
Addison's disease	P450c21, P450scc, P450c17
Hypoparathyroidism	Calcium-sensing receptor, NALP5[b]
Minor manifestations	
Ovarian failure	P450c17, P450scc
Testicular failure	TSGA10[b], TGM4[b]
Type 1 diabetes	GAD65[b], insulin, 1A-2
Hashimoto thyroiditis	Thyroid peroxidase, thyroglobulin
Graves' disease	TSH receptor, thyroid peroxidase
Hypopituitarism	TDRD6[b]
Autoimmune hepatitis	CYP1A2[a], CYP2A6, AADC[a], LKM, TPH[a]
Autoimmune gastritis/ pernicious anaemia	H/K-ATPase of gastric parietal cells, intrinsic factor
Coeliac disease	Endomysial, TTG
Gastrointestinal dysfunction	TPH[a], histidine decarboxylase, GAD65
Vitiligo	Melanocytes, SOX9 and SOX10, AADC[a]
Alopecia	Tyrosine hydroxylase[a]
Tubulo-interstitial nephritis	Tubular basement membrane[b]
Pulmonary disease	KCNRG[b]

P450c21, steroid 21-hydroxylase; P450scc, cholesterol side-chain cleavage enzyme; P450c17, steroid 17α-hydroxylase; NALP5, NACHT leucine-rich repeat protein 5; IFN, interferon; IL, interleukin; TSGA10, testis-specific gene 10; TGM4, prostate-specific enzyme transglutaminase 4; GAD65, glutamic acid decarboxylase 65; 1A-2, tyrosine phosphatase-like protein 1A-2; TDRD6, tudor domain containing 6; CYP1A2, cytochrome P450 1A2; CYP2A6, cytochrome P450 2A6; AADC, aromatic L-amino acid decarboxylase; LKM, liver–kidney microsomal; TPH, tryptophan hydroxylase; TTG, tissue transglutaminase; SOX, transcription factors; KCNRG, potassium channel regulator.

[a] Almost exclusive to APS1 and thus helpful in differentiating APS1 from other autoimmune diseases [4, 5, 9, 11, 17, 28–30, 33–36].

[b] Not strongly associated with the disease component in APS1 – thus relevance unclear and therefore of poor predictive value [31].

essential to prevent fatalities and DNA screening for *AIRE* mutations or serology for anti-interferon antibodies should be performed in any subjects with an atypical presentation. Mutational analysis has aided early diagnosis but there are a large number of possible mutations and only the commonest two are routinely screened in the UK. Thus, APS1 is not excluded by negative routine DNA analysis and the presence of one abnormal allele in a child with a major or minor manifestation makes the diagnosis highly likely. In view of this, screening for autoantibodies to IFN-α and IFN-ω, which are sensitive and specific, has now become the gold standard tool and has an essential role in diagnosing atypical APS1 [9, 14].

Although there is often no clinical value in DNA analysis in subjects with two or more cardinal features, the molecular findings in a proband will be of value in counselling and for screening siblings. Siblings should always be examined, as one of the cardinal manifestations or a definite ectodermal component is clinically diagnostic.

Early diagnosis is essential, not only to pre-empt the onset of serious complications such as hypocalcaemia and adrenal failure, but also to allow consideration of pre-emptive immunomodulation, which may alter the natural history of the disease and reduce the effects of long-term complications. It also enables families to receive accurate and timely genetic counselling.

Follow-up

Patients with established APS1 and those with one or more suspicious features need standard endocrine follow-up to prevent life-threatening situations. The search for antibodies predicting new diseases can help early diagnosis (Table 11.3). Each visit requires a thorough history and examination, particularly for oral mucocutaneous candidiasis and signs of evolving adrenal insufficiency, such as salt craving or a postural change in blood pressure. Blood should be taken for basal hormone, haematological and biochemical markers and an annual antibody screen performed (Table 11.4). This, together with a high index of clinical suspicion, allows earlier diagnosis and treatment.

An early diagnosis of Addison's disease is of particular importance. Individuals are all at risk and need annual measurement of ACTH [3] and plasma renin activity. Adrenal failure can evolve rapidly in APS1 and annual assessment may not be sufficient to prevent acute presentation. The patient, immediate family and primary healthcare team must be made aware of the signs and symptoms of adrenal failure. Postural blood pressure and serum electrolytes should be determined at each clinic visit, together with annual screening for 21-hydroxylase autoantibodies. If adrenal antibody positive, 6 monthly electrolytes, early morning cortisol, ACTH concentrations and renin activity should be measured to monitor

Table 11.4 Investigations recommended in the routine follow-up of APS1 patients to attempt to identify early development of new complications.

Disease component	Laboratory investigation
Major manifestation	
Addison's disease	U&E, ACTH, plasma renin activity, early morning cortisol, Synacthen test (as indicated)
Hypoparathyroidism	Serum calcium, phosphate and magnesium, parathyroid hormone
Minor manifestation	
Hypogonadism	Gonadotropin levels, testosterone/oestradiol
Type 1 diabetes	Glycosylated haemoglobin, random glucose, glycosuria
Autoimmune thyroid disease	fT3, fT4 and TSH
Hypopituitarism	IGF1
Autoimmune hepatitis	Liver function tests
Atrophic gastritis/pernicious anaemia	FBC[a]
Pancreatic exocrine insufficiency	Faecal elastase
Hypo/asplenism	FBC[a], blood smear[b]
Renal involvement	Electrolytes, urinalysis
Iatrogenic nephrocalcinosis (if on calcium supplements)	Urine calcium/creatinine ratio, annual renal ultrasounds
Others	
Annual autoantibody screen	
Regular ophthalmic review	
Regular dental check-ups	

[a] The presence of anaemia on full blood count (FBC) results needs further investigation with ferritin, transferrin and serum iron levels if the anaemia is microcytic and vitamin B_{12} levels if macrocytic.
[b] A blood smear indicating hypo/asplenism (Howell–Jolly bodies, anisocytes, poikilocytes, target cells and burr cells) and/or the presence of thrombocytosis needs follow-up with an abdominal ultrasound to assess spleen presence and size.

trends, with a Synacthen test performed if there are any clinical or biochemical concerns.

More severe and atypical infections are seen in APS1 patients; therefore, any infectious complications in APS1 need rapid attention and additional surveillance [22]. Good dental hygiene is important and should include monitoring for oral CMC, oral cancer and enamel hypoplasia. Difficulty in swallowing or retrosternal pain should lead to endoscopy. Regular ophthalmology review and monitoring of respiratory function should also be part of routine follow-up.

Treatment

Treatment of the individual disorders is not different from treating patients with the isolated disorders, except that polypharmacy is the rule and that malabsorption may complicate therapy. Adherence to treatment can be a challenge, particularly in teenagers. Immunosuppressive treatment with glucocorticoids can complicate matters and, in more serious situations, such as fulminant hepatic failure, combinations of immunosuppressants are often required. Psychological support is needed for many patients for whom high rates of depression, social isolation, alcoholism and substance misuse are reported, particularly as patients reach adulthood.

Mucocutaneous candidiasis is treated with local and/or systemic antifungal drugs, dental care and oral hygiene with oral surgical follow-up for refractory cases. Aggressive treatment of oral candidiasis is important because of the risk of oral carcinoma and suspicious oral lesions should be biopsied. Intermittent prophylactic courses of topical oral treatment are recommended. Fluconazole or ketoconazole is indicated if topical treatments fail. The use of IV polyene antifungals and the newer echinocandins should be used only for systemic candidal infections due to their side effects. Itraconazole or miconazole is preferred for nail candidiasis but requires a course of 4–6 months [8]. All oral antifungal agents can cause transient elevation of liver enzymes and occasionally hepatitis, so close monitoring is required. Ketoconazole is a global P450 cytochrome inhibitor and so can precipitate decompensation in patients with marginal adrenal reserve. Conversely, prolonged treatment with itraconazole in a patient taking oral glucocorticoid replacement

medication may lead to Cushing's syndrome as steroid metabolism is inhibited.

Patients with adrenal failure and/or hypoparathyroidism are managed as described in Chapters 9 and 10, respectively. Serum calcium concentrations in APS1 patients appear to be labile compared with non-APS1 hypoparathyroidism, and serious hypercalcaemia can occur despite previous long periods of normo- or hypocalcaemia. Although standard doses of alfacalcidol (25–50 ng/kg/day) or calcitriol (15 ng/kg/day) can be used initially, patients with APS1 often require much larger doses to maintain eucalcaemia (3–5 μg/day being not unusual). This is presumed to be due to malabsorption and the intermittent nature of this can lead to episodes of marked hypercalcaemia with rapid onset of renal impairment. Although alfacalcidol is generally preferred as the first-line treatment in children due to more experience with its use and appropriate formulations being available, calcitriol, which is unlicensed in children in the UK, may have a role if severe liver disease is present.

Our practice is to monitor serum calcium and phosphate concentrations 8–12 weekly with regular determinations of urinary calcium excretion. Treatment with vitamin D analogues often leads to hypercalciuria, so serum calcium concentrations need to be maintained around the lower end of the normal range or even more commonly, mildly hypocalcaemic (2.0–2.2 mmol/L total serum calcium), aiming to keep urine calcium concentrations in the normal range. The vicious cycle of hypocalcaemia and malabsorption (as hypocalcaemia leads to steatorrhoea) can usually be broken by an increased oral dose but parenteral therapy may be required. Refractory cases may benefit from monthly intramuscular (IM) injections of calciferol in addition to the daily use of oral alfacalcidol to maintain basal concentrations. Hypomagnesemia may contribute to resistance and require treatment and monitoring of vitamin D concentrations with appropriate supplementation is recommended.

Owing to the prevalence of nephrocalcinosis, our practice is to perform occasional (3 yearly) renal ultrasonography in subjects with hypoparathyroidism, taking the additional opportunity to assess the gall bladder and the size of the spleen. This should be increased to annually if there are suspicions of nephrocalcinosis. In patients with adrenal insufficiency, alteration of the cortisol dose, for example, at times of stress, may lead to an alteration in calcium absorption. Also of note is that unexplained hypercalcemia may be the first sign of the development of adrenal failure.

Female hormonal substitution with oestrogen in puberty is required in those with delayed or arrested puberty. It is recommended that pregnancy not be delayed and there are reports of successful embryo donation in women affected with early menopause due to APS1. Testosterone in affected males should be replaced as necessary.

Some of the other serious non-endocrine manifestations of APS1, such as autoimmune hepatitis and severe intestinal dysfunction, require immunosuppressive treatment. In the past there were concerns that immunosuppressive therapy might increase the risk of *Candida*-related cancer and predispose the patient to generalized candidal infection but, with the recognition that CMC is an autoimmune disease, this is no longer felt to be an issue and has not been observed clinically. Modern immunosuppressants are well tolerated and can lead to a dramatic improvement in many aspects of APS1, not just autoimmune hepatitis and intestinal dysfunction. There are increasing suggestions that early initiation of immunosuppression with appropriate monitoring is appropriate in severely affected cases.

The use of immunosuppressive treatment in APS1 has been reported mainly in the context of managing autoimmune hepatitis, renal involvement and intestinal dysfunction; an associated improvement in serum calcium concentrations has been reported in some cases. A range of agents, either alone or in combination, has been tried including prednisolone, methotrexate, cyclosporine A, azathioprine, mycophenolate mofetil (MMF) and tacrolimus. MMF has been particularly successful as a 'steroid-sparing' agent. Dramatic improvements with immunosuppression, particularly with respect to malabsorption, autoimmune hepatitis, growth, management of hypoparathyroidism, alopecia and keratitis, have been reported but other studies report more variable results, particularly with respect to renal involvement [21]. Failure of one immunosuppressant does not necessarily infer failure of others [39] and a trial of a different agent may well be indicated. For example, the successful use of rituximab (a monoclonal antibody targeting B-cell lymphocytes) in a young patient with bronchiolitis has been reported [40]. The decision to treat with immunosuppressants should be made on an individual basis and tailored to the specific issues of a particular patient. Immunosuppressive treatment is not always successful and liver transplantation has occasionally been needed in APS1-associated hepatitis.

Milder diarrhoea may respond to gut motility-reducing agents such as loperamide. Oral bile acid replacement therapy, low fat diets or medium-chain triglyceride diets may help with fat malabsorption in patients with steatorrhoea resulting from cholecystokinin deficiency. Pancreatic exocrine insufficiency is often observed and is treated with oral pancreatic enzyme replacement and dietary modification.

Keratitis requires treatment with local glucocorticoid and topical vitamin A has been found to prevent corneal ulcers. Successful corneal transplantation has also been reported.

As splenic atrophy is a common component, all APS1 patients should receive polyvalent pneumococcal vaccine with measurement of antibody response 6–8 weeks later. Non-responders or those who are asplenic should receive prophylactic daily antibiotics.

Prognosis

Many patients feel chronically unwell and the physical and psychological impact of the multiple problems should not be underestimated; alcohol intake and smoking is greatly increased in this patient group, as is malignancy [5]. Despite improved survival, mortality rates are still high at 10–20% and recent reviews in Finland and Norway have found the average age of death to be 34 years (range of 3–64 years) [5, 12] from a variety of causes including adrenal crisis, fulminant hepatic failure, oral and oesophageal carcinoma secondary to CMC, septicaemia, hypocalcaemia, complications of kidney failure, multi-organ failure, diabetic ketoacidosis and alcoholism [3, 8, 11].

Around 3% die before the diagnosis of APS1 has been made, with adrenal failure the most likely cause. Depression and suicide is high among this patient group as the disease poses a great psychological burden with the constant risk of developing life-threatening complications, disfiguring disease components and the requirement for multiple medications. Working capacity may be maintained in subjects with a limited number of manifestations but many are significantly incapacitated [11, 12].

Summary

The clinical presentation of APS1 is very variable. Diagnosis can be difficult initially when only one manifestation is present and it often takes years for others to appear. Increased awareness of the condition combined with analysis of specific autoantibodies and mutational analysis of the *AIRE* gene should help to diagnose this condition earlier and help prevent serious complications and fatalities.

Autoimmune Polyendocrinopathy Syndrome Type 2 and Associated Disorders

Definitions

APS2 is defined by the presence of primary adrenocortical insufficiency with either autoimmune thyroid disease or T1D in the same individual. An autoimmune origin of all the major components should be demonstrated for the correct diagnosis. The association of autoimmune Addison's disease and autoimmune thyroid disease is known as Schmidt syndrome and the association of Addison's disease with T1D is called Carpenter syndrome. Other endocrine and non-endocrine autoimmune disorders occur with increased frequency in these individuals and their families [2].

Table 11.5 Classification of APS3.

Autoimmune thyroid disease plus:	
Autoimmune endocrinopathy excluding Addison's disease, e.g. type 1 diabetes, premature ovarian failure, lymphocytic hypophysitis	3A
Autoimmune gastrointestinal disease, e.g. pernicious anaemia, coeliac disease, autoimmune hepatitis	3B
Skin or neurological manifestations, e.g. alopecia, vitiligo, myasthenia gravis	3C
Connective tissue disease, e.g. SLE, rheumatoid arthritis, Sjögren syndrome	3D

SLE, systemic lupus erythematosus.

APS3 is defined as the association between autoimmune thyroid disease and an additional autoimmune disease other than Addison's disease [10]. Many clinical combinations can be found and it can be subdivided into 3A to D, depending on the associated conditions (Table 11.5) [10]. Some authors use the term APS4 to encompass an association of autoimmune diseases not falling into the categories APS1–3. Many of these patients develop more classical APS2/3 manifestations later and, as this is an extremely heterogeneous group of patients, a description of the individual components may be helpful.

APS2

Clinical Features and Course

APS2 has an estimated prevalence of 4–5/100,000 [41, 42]. Clinical presentation can be at any age but is most frequently in early adulthood, with a peak onset in the fourth decade. It is recognized less commonly in children and adolescents. It affects both sexes with a female–male ratio of 3 : 1 [41].

Major Manifestations

Addison's disease is present in 100% of cases, autoimmune thyroid disease in 70–90%, and T1D in 20–50% [1, 9, 39, 40]. Only about 10% have the complete triad [9, 40]. Approximately two-thirds of patients with autoimmune Addison's disease have APS2. Adrenal failure is the first endocrine abnormality in around 50% but several minor APS2 components are often present when the diagnosis of adrenal failure is made: T1D already exists in

around 20% and autoimmune thyroid disease in around 30%. Either component may have been present for more than 20 years before the diagnosis of adrenal failure.

Autoimmune thyroid disease encompasses Hashimoto's thyroiditis, atrophic hypothyroidism, Graves' disease and post-partum thyroiditis. Hypothyroidism is commoner than Graves' disease but Graves' disease tends to present at a younger age in the context of APS2.

Delayed diagnosis and preventable deaths still occur in patients with undiagnosed adrenal failure. Signs and symptoms are often vague and non-specific until an adrenal crisis ensues. Low morning serum cortisol concentrations and electrolyte abnormalities (hyponatraemia and hyperkalaemia) are late changes, occurring at or just before the onset of clinical adrenal insufficiency. Hyperpigmentation may be observed but may be absent in fair- or red-headed subjects. Adrenal insufficiency may present as hypoglycaemic seizures in children.

In those who already have T1D, deterioration of glycaemic control with recurrent hypoglycaemia and a decrease in total insulin requirements may point to evolving adrenal insufficiency. The onset of autoimmune hyperthyroidism or thyroxine replacement for newly diagnosed hypothyroidism leads to enhanced cortisol clearance and can precipitate an adrenal crisis in subjects with subclinical adrenocortical failure. Clinicians should maintain a high degree of alertness for underlying adrenal failure before initiating thyroid hormone replacement. Conversely, cortisol inhibits thyrotropin release, so thyroid-stimulating hormone (TSH) concentrations are often high at the initial diagnosis of adrenal insufficiency (typically 5–10 mU/L) but return to normal after initiation of glucocorticoid replacement in the absence of coexistent thyroid disease. Adrenal insufficiency can mask the hyperglycaemia of T1D.

Latent **a**utoimmune **d**iabetes in **a**dults (LADA) is a recognized component of APS2: it develops in adulthood with diabetes-associated autoantibodies and a delay from diagnosis in the need for insulin therapy. The clinician needs to remain vigilant for the development of other autoimmune conditions regardless of the age of the patient.

Minor Manifestations

These are listed in Table 11.6 together with their frequency. All these associated autoimmune disorders are present at lower frequency in APS2 compared with APS1 and they are usually associated with their respective immunological markers. Primary hypogonadism is one of the commonest minor manifestations in APS2/3 females, premature ovarian failure leading to secondary amenorrhoea in around 10% of women under 40 years. Testicular failure is very rare. Pituitary involvement is very occasionally seen in APS2/3, with lymphocytic hypophysitis leading to empty sella syndrome, panhypopituitarism or isolated failure of any of the anterior pituitary hormones.

Table 11.6 Minor manifestations frequently associated with APS2.

Minor manifestation	Frequency (%)
Pernicious anaemia	1–25
Gonadal failure	
Females	3.5–10
Males	1–2
Vitiligo	4–12
Alopecia	2–5
Autoimmune hepatitis	4
Malabsorption (including coeliac disease)	1–2
Sjögren syndrome	1
Neoplasias	3
Rarer manifestations	
Endocrine	*Neurological*
Pituitary involvement	Myositis
Hypophysitis	Myasthenia gravis
Empty sella syndrome	Neuropathy
Late-onset hypoparathyroidism	Stiff man syndrome
Gastrointestinal	*Others*
Ulcerative colitis	Sarcoidosis
Primary biliary cirrhosis	Serositis
Dermatological	Selective IgA deficiency
Granuloma annulare	Idiopathic heart block
Dermatitis herpetiformis	Idiopathic thrombocytopenia Purpura
	Rheumatoid arthritis

Hypoparathyroidism is very rare in APS2/3 (*cf*APS1) but, if hypocalcaemia does occur, PTH and vitamin D concentrations should be checked and coeliac disease excluded. Hypoparathyroidism has been described in a few adult patients with parathyroid-suppressing antibodies [33, 43] often coexisting with autoimmune thyroid disease. Autoimmune hypoparathyroidism in childhood is almost pathognomic of APS1.

Incomplete APS2

Patients with autoimmune thyroid disease or T1D and adrenal autoantibodies in the serum or patients with Addison's disease and either thyroid or islet cell

autoantibodies but no overt biochemical dysfunction are sometimes classified as incomplete APS2 [10]. Self-evidently, these patients may develop APS2 in the future. Annual screening by ACTH and renin measurement, together with education about the likely presentation of adrenal failure, is important in these circumstances. About 30% of subjects with positive adrenal antibodies progress to adrenal failure over a 6 year period [44]. Patients with either autoimmune thyroid disease or T1D alone who have a sibling with APS2 are also classified by some as having incomplete APS2 because of their possible higher risk of adrenal failure [10].

APS3

APS3 is the association between autoimmune thyroid disease and autoimmune disorders other than Addison's disease. Hashimoto's thyroiditis is the commonest form of autoimmune thyroid disease, although Graves' disease and post-partum thyroiditis are also seen. Autoimmune thyroid diseases tend to increase in incidence in the teenage years, with a peak in the fourth decade for Graves' disease and in the fifth and sixth decades for autoimmune hypothyroidism. Autoimmune thyroid disease is most commonly isolated and polyglandular involvement in the form of APS3 or APS2 is rare (<5%). Only 1% of patients with isolated autoimmune thyroid disease have adrenal autoantibodies (with risk of APS2), whereas 3–5% have either pancreatic islet autoimmunity or clinical T1D [45]. The presence of thyroid and glutamic acid decarboxylase antibodies (GADA) predicts the development of hypothyroidism in young children [46].

Autoimmune thyroid disease is more commonly associated with pernicious anaemia, vitiligo, alopecia, myasthenia gravis and Sjögren syndrome and autoimmune thyroid disease should be sought prospectively in patients with these conditions. Around 30% of subjects with vitiligo have another autoimmune disorder, with autoimmune thyroid disease and pernicious anaemia being the most common. Many patients with vitiligo are asymptomatic and other autoimmune diseases are diagnosed only by prospective screening, including evaluation of autoantibody status. Up to 15% of patients with alopecia and nearly 30% of those with myasthenia gravis have autoimmune thyroid disease.

Genetics

APS2 is a genetically complex and multifactorial disease. It aggregates in families and appears to show an autosomal dominant pattern of inheritance with incomplete penetrance in some. Susceptibility is determined by multiple genetic loci that interact with environmental factors. Only two genes have shown consistent association with APS2: *HLA* and *CTLA4*. Of these, *HLA* appears to have the strongest gene effect [1]. Four further genes have been associated with AAD and may play a role in APS2 development, namely, *PTPN22*, *BACH2*, *CYP27B1* and *GPR174* [47–50].

HLA and APS2

Many of the component disorders in APS2, including autoimmune thyroid disease, T1D, Addison's disease, coeliac disease, myasthenia gravis, selective IgA deficiency and dermatitis herpetiformis, are associated with the same extended *HLA* haplotype: *HLA-A1*, *HLA-B8*, *HLA DR3*, *DQA1*0501*, *DQB1*0201* (*DQ2*). Thus, unsurprisingly, *HLA DR3*, *DQB1*0201* is associated with APS2. T1D and, to a lesser extent, Addison's disease also show association with *HLA DR4*, *DQA1*0301*, *DQB1*0302* (*DQ8*) and *HLA DR5* shows association in patients with a combination of Addison's disease and autoimmune hypothyroidism [10]. Some 35% of individuals with T1D are heterozygous for the *HLA DR3/DR4* combination, with about 50% of children having this combination of haplotypes developing T1D under 5 years of age. Although specific *HLA* haplotypes influence susceptibility to APS component disorders, others appear to be protective. The haplotype *DR2* (*DRB1*1501*), *DQA1*0102*, *DQB1*0602* appears to provide dominant protection against T1D, even in the presence of insulin autoantibodies. Patients with P450c21 autoantibodies and *DRB1* 0401* and *DRB1*0402* appear to progress to adrenal failure less often.

CTLA4 and APS2

CTLA4 encodes an important negative regulator of T-cell activation expressed on the surface of activated T lymphocytes. Alleles of *CTLA4* have been linked primarily to autoimmune thyroid disease, both Graves' disease and Hashimoto's thyroiditis, but there is also a weaker association with T1D. Addison's disease (either isolated or as part of APS2) has been shown to be associated with *CTLA4* alleles in some populations including a subgroup of patients carrying *HLA DQA1*0501* [51, 52].

PTPN22, CYP27B1, GPR174 and BACH2

The *PTPN22* gene encodes lymphoid tyrosine phosphatase (LYP), which plays a key role in early T-cell activation. Association with a functionally significant tryptophan for arginine variant in LYP has been found in a mixed UK cohort of AAD and APS2 subjects [53] and in Norwegian subjects [47] but this has not been replicated in all cohorts.

CYP27B1, the gene encoding vitamin D 1α-hydroxylase, is involved in immune regulation and cell proliferation and small studies have shown association of *CYP27B1* alleles with AAD [49].

GPR174 is a G-protein-coupled receptor located at Xq21 that is widely expressed in lymphoid tissues. An association was found between a single-nucleotide polymorphism, rs3827440, encoding Ser162Pro in this X-linked gene and Addison's disease in a UK cohort [50]. This same polymorphism is also associated with Graves' disease.

BACH2 is a transcription factor expressed in B lymphocytes and encoded by a gene on the long arm of chromosome 6 distinct from the HLA region. Variants within *BACH2* have been associated with T1D, Graves' disease, coeliac disease, multiple sclerosis and Addison's disease [48].

The association of the component disorders in APS2 is therefore partly related to the shared susceptibility alleles including *HLA*, *CTLA4*, *PTPN22*, *CYP27B1* and *BACH2* conferring risk to the different diseases. It is likely that there is a complex interaction between these variants and other as yet unidentified loci and environmental factors.

Autoantibodies and Pathogenesis

The pathogenesis of autoimmunity in APS2 is considered as a multifactorial or complex genetic trait similar to that of the individual disease components. There are several hypotheses to explain why autoimmunity occurs against multiple organs in individuals with APS.

It may result from a shared epitope(s) between an environmental agent and a common antigen present in several endocrine tissues or the organs derived from the same germ layer expressing common germ layer-specific antigens that could serve as targets for the autoimmune response in APS. More likely is a subtle thymic defect of negative selection of autoreactive T cells caused either by a defect in T-cell apoptosis or by a problem in presentation of self-antigens. This may be most severe for low-abundance, specialist antigens, such as those needed for the biosynthesis, secretion and regulation of the various hormones. Defects in CD4+CD25+ regulatory T-cell suppressor function and impaired caspase-3 expression by peripheral T cells have also been demonstrated. Thus loss of peripheral suppression and/or defective peripheral apoptosis could be involved in the pathogenesis of this syndrome.

At the onset of autoimmune adrenal failure, adrenal cell autoantibodies or P450c21 autoantibodies are detectable in >90% of patients [10, 42]. P450c21 has been identified as the major adrenal antigen in autoimmune adrenalitis and these antibodies are present in 80–90% of patients with disease duration under 15 years, declining to 60% with disease duration over 15 years. These P450c21 autoantibodies are highly specific, being found in only 0.5% of healthy subjects and those with other autoimmune diseases. Some 40–50% of patients with such adrenal autoantibodies have abnormal ACTH stimulation tests. Thus, P450c21 autoantibodies have a high predictive value for clinical Addison's disease. Spontaneous disappearance of adrenal antibodies has been reported in up to 20% of cases [42] but disease is permanent in patients who have an abnormal ACTH stimulation test.

Other steroid-producing cell autoantibodies (SCA) such as P450c17 and P450scc are present in 20–30% patients with Addison's disease and are more frequent in females than males. There is a strong association between the presence of SCA and ovarian failure in women with APS2/3 but SCA are extremely rare in women with ovarian failure with no signs of adrenal autoimmunity. Because of the shared antigens of the steroidogenic enzymes, adrenal autoimmunity is more common (>10%) in those subjects with established gonadal failure.

Autoimmune thyroid disease and T1D are frequent components of APS2. Thyroperoxidase (TPO) and thyroglobulin (TG) are the major thyroid antigens. In Hashimoto's thyroiditis, TPO autoantibodies are found in 90–100% and TG autoantibodies in 60–70%. They are both also frequently found in Graves' disease, where TSH receptor autoantibodies are found in around 90% of cases [43]. Many patients with thyroid autoantibodies but normal TSH progress very slowly to clinical disease.

Islet cell autoantibodies are found in around 80% of new-onset T1D patients [42]. The main islet autoantigens are insulin, GAD65 and the tyrosine phosphatase-related protein IA-2. Among recently diagnosed subjects with T1D, the prevalence of antibodies to insulin and IA-2 is dependent on age, being most frequent in children and adolescents but <30% with adult onset or LADA. The frequency of antibodies to GAD65 is 70–80% and is not influenced by age; this therefore gives the highest diagnostic sensitivity in LADA. In one investigation, all APS2 patients with T1D were positive for GAD65 antibodies but only 54% of those with antibodies had T1D. By comparison, IA-2 antibodies are less sensitive but more specific for T1D.

Gastric parietal cell autoantibodies are found in about 90% of patients with chronic autoimmune gastritis or pernicious anaemia and in 30% of their non-anaemic first-degree relatives. The major autoantigen is gastric H/K-ATPase. Around 70% of patients with pernicious anaemia are also positive for intrinsic factor autoantibodies that block the binding of vitamin B_{12} to intrinsic factor [43]. TTG is the major autoantigen in coeliac disease. IgA TTG antibodies are more specific for coeliac disease than IgG but both have a high diagnostic sensitivity and specificity. There is good correlation between endomysial autoantibodies and TTG antibodies [43].

Diagnosis and Follow-up

Once APS2/3 is suspected, a full assessment of endocrine function is needed. The number of disorders that will develop and the age at which they will present is unpredictable, so long-term follow-up is needed. A high index of clinical suspicion needs to be maintained, particularly in subjects who have yet to develop adrenal failure or diabetes. Pre-symptomatic recognition of autoimmune disease minimizes associated morbidity and mortality.

There is a clear link between the presence of organ-specific autoantibodies and the progression to disease, although there is often an asymptomatic latent period of months or years. The absence of autoantibodies does not exclude the risk of a disease component.

In any patient with clinical and biochemical signs of adrenal insufficiency, determination of P450c21 autoantibodies demonstrates the autoimmune nature of the disease [43]. An aetiological diagnosis should be sought in all subjects but the presence of autoimmune disorders in family members is suggestive of autoimmunity. Around 60% of patients with Addison's disease have one or more associated autoimmune disease [54] and so screening for other endocrine disorders, particularly autoimmune thyroid disease and T1D, is important. At diagnosis, screening for TPO and GAD65 autoantibodies is worthwhile. If negative, this should be repeated occasionally, perhaps every 2–3 years. In children or adolescents with Addison's disease, determination of insulin and IA-2 autoantibodies is a sensitive predictor of T1D, particularly if both autoantibodies are present. If these β-cell autoantibodies are found, an assessment of fasting blood glucose, HbA1c, and, in some cases, an oral glucose tolerance test are required.

The determination of thyroid function should be carried out at least annually for early recognition of thyroid disease in all subjects with T1D and Addison's disease. The determination of P450c17 and P450scc antibodies in females with Addison's disease and APS2 may identify subjects at high risk from primary hypogonadism before gonadotropins become elevated. Such subjects may be suitable for cryopreservation of ovarian material.

The determination of P450c21 autoantibodies should be performed in children presenting with T1D as positive adrenal autoantibodies are highly predictive of future adrenal insufficiency [38]. In subjects with P450c21 autoantibodies, an ACTH stimulation test, determination of electrolytes and plasma renin activity enables identification of patients with preclinical adrenal dysfunction. If normal, the ACTH stimulation test should be repeated yearly with interval determination of postural blood pressure and electrolytes. A single measurement of ACTH as opposed to ACTH stimulation may also be a useful way of detecting failing adrenal gland function [55]. Regardless of antibody status, patients with persistent or worsening symptoms after treatment of autoimmune thyroid disease and subjects with T1D who have brittle control or persistent lethargy or those with unexplained vague symptoms should be screened biochemically for Addison's disease.

An increased frequency of IgG–TTG antibody has been found in T1D children but the prevalence in adult Addison or T1D patients is the same as in the healthy population. Thus, they should be included in APS2/3 screening of children but limited in adults to cases with clinical or laboratory signs of malabsorption. Positive TTG antibodies in children usually require follow-up with an intestinal biopsy to confirm the diagnosis of coeliac disease. The role of screening with TTG antibodies and intestinal biopsy in the individual with T1DM who is healthy with good glycaemic control is unclear.

The predictive value of gastric parietal cell or intrinsic factor autoantibodies for autoimmune gastritis and pernicious anaemia is limited by the frequent occurrence of these in healthy first-degree relatives and in the general population (~5–10%). A blood count to detect macrocytosis is a more useful routine investigation, although neurological features of vitamin B_{12} deficiency can be present in the absence of anaemia. Thus, vitamin B_{12} concentrations should be measured if clinically suspected.

Screening for APS2-associated disorders should also be performed in young women with primary or secondary amenorrhoea or premature ovarian failure and in young patients with vitiligo. As APS2 shows strong familial tendencies, family members should also be checked for features of associated endocrine conditions.

Management

Hormone replacement or other therapies for the component diseases of APS2 are similar whether the disease occurs in isolation or in association with other conditions and disorders and should be started at diagnosis but some combinations of disease require specific attention. Most importantly, levothyroxine therapy for hypothyroidism can precipitate a life-threatening adrenal crisis in a patient with untreated and unsuspected adrenal insufficiency. Thus, to avoid adrenal crisis, clinicians should maintain a high degree of suspicion for coexisting adrenal failure in subjects who are hypothyroid. Hyperthyroidism increases cortisol clearance so, in patients with adrenal insufficiency who have unresolved hyperthyroidism, glucocorticoid replacement should

be at least doubled until the patient is euthyroid. Hyperthyroidism is associated with a reduction in insulin sensitivity and a deterioration in glycaemic control in the patient with T1D while decreasing insulin requirements or increasing occurrence of hypoglycaemia in T1D can be one of the earliest indications of adrenocortical failure.

Prognosis

Mortality in patients with primary adrenal insufficiency is elevated about twofold compared to the background population and around fourfold in patients with Addison's disease and diabetes when compared to those with diabetes alone [56]. Life expectancy is often reduced as a consequence of unrecognized adrenal crisis but infectious disease, cardiovascular disease and cancer also appear to be increased. Despite adequate hormonal replacement, quality of life is often impaired in these patients, with predominant complaints being unpredictable fatigue, lack of energy, depression and anxiety. It has been shown that the number of patients receiving disability pensions is two- to threefold higher than the general population in certain countries. Clinical trials are now exploring the potential for adrenal gland regeneration using agents such as ACTH and immune modulators like rituximab in part because of these chronic health issues [57, 58].

Summary

A high index of suspicion needs to be maintained whenever one organ-specific autoimmune disorder is diagnosed in order to prevent morbidity and mortality from the index disease as well as associated diseases. Further definition of susceptibility genes and autoantigens, as well as a better understanding of the pathogenesis, is required to improve the diagnosis and management of these patients.

Other Single Gene Disorders Associated with Immune Dysregulation and Autoimmune Endocrinopathies

Polyendocrinopathy, particularly if early onset, can be a manifestation of an expanding number of recognized immune dysregulation syndromes. There may be considerable overlap with APS 1 but immunodeficiency is a more prominent feature in these disorders and the most common endocrinopathy is usually either autoimmune thyroid disease or T1D. The clinical presentation of these immune dysregulation syndromes is highly variable; the initial phenotype tends to be relatively narrow but has expanded as patient cohorts have been screened. Patients previously undergoing haematopoietic stem cell transplantation (HSCT) for immune dysregulation syndromes have tended to have a more severe phenotype and be more unwell as a result of these trends. Patients diagnosed sooner will be healthier and have an improved outcome following HSCT. Recent series suggest that survival rates for HSCT may be 90% or more.

Immune Dysregulation, Polyendocrinopathy and Enteropathy (X-linked) (IPEX) Syndrome

IPEX is a rare and devastating X-linked condition of male infants, affecting immune regulation and resulting in multiple autoimmune disorders. The first feature is commonly early-onset intractable diarrhoea and failure to thrive due to autoimmune enteropathy occurring around 3–4 months of age. T1D and autoimmune hypothyroidism develop in the first year of life in around 90 and 50% of males, respectively. Additional clinical features include eczema, autoimmune haemolytic anaemia, autoimmune thrombocytopenia, recurrent infections, lymphadenopathy, membranous nephropathy, facial myopathy and striking growth retardation. Other autoimmune features are less frequent [59]. Sepsis may result from a primary defect in immune regulation but is exacerbated by autoimmune neutropenia, immunosuppressive drugs, malnutrition, enteropathy and eczema.

The condition is heterogeneous in its presentation, with the occasional case not presenting until later childhood or adulthood. Any disease component can present first, including diabetes and eczema. There are no estimates of incidence but it is likely to be underdiagnosed because of the clinical variability in presentation and the presence of frequent new mutations. Intermittent eosinophilia and raised IgE concentrations are found in many patients but there is an absence of any other consistent features of immunodeficiency. The presence of autoantibodies appears to be variable.

The most consistent pathological finding is total villous atrophy of the small intestine, with inflammatory cell infiltration of the lamina propria. Diagnosis relies on the clinical presentation, family history and elimination of other diagnoses with similar presentations. Genetic screening has proved useful in many cases. There is a high mortality in these infants, many succumbing to the untreatable diarrhoea, malnutrition and superimposed infections by 24 months of age. Survival into adolescence is occasionally seen with the use of aggressive immunosuppression and parenteral feeding, although symptoms are rarely entirely relieved [59, 60]. There are an increasing number of reports of successful bone marrow transplantation in these infants with associated restoration of T regulatory $CD4^+$ function [61].

IPEX was first reported more than 20 years ago in a large family with typical X-linked recessive inheritance [62]. IPEX is mediated by an abnormality in $CD4^+$ T-cell regulation, with evidence for increased T-cell activation and overproduction of cytokines. By recognition of a similar phenotype in a murine model, mutations in the *FOXP3* gene, located at Xp11, encoding a transcription factor belonging to the forkhead/winged-helix family, were found in IPEX boys. An increasing number of mutations have been reported, mainly in the coding region of *FOXP3*, although mutations in the regulatory region have also been found. *FOXP3* is specifically expressed in naturally arising $CD4^+CD25^+$ regulatory T cells and appears to convert naïve T cells to this regulatory phenotype. Thus, *FOXP3* is a critical regulator of $CD4^+CD25^+$ T-cell development and function [63]. Although female carriers of *FOXP3* mutations appear to be healthy, a small number of cases of an IPEX-like syndrome have been reported in families with affected girls in whom no mutation was found [60]. It is likely that there may be an autosomal locus accounting for the problem in some families, and mutations in the IL-2 receptor subunit CD25 have been shown to cause a similar syndrome [64]. This genetic heterogeneity may explain some of the clinical variation seen in this syndrome but no obvious genotype–phenotype relationship has been identified. Other modifying genes, such as *HLA*, as well as environmental factors may influence disease evolution.

Autoimmune Lymphoproliferative Syndrome (ALPS)

ALPS was first described in 1967, although the aetiology and pathogenesis of the condition were unknown at the time [65]. Onset is usually in the first 2 years of life and the characteristic feature in all cases is massive generalized lymphadenopathy. Hepatosplenomegaly and haematological autoimmunity (haemolytic anaemia and thrombocytopenia) are also frequent manifestations. Other autoimmune conditions, including thyroid autoimmunity and T1D, have occasionally been reported.

Mutations of the Fas receptor or of its ligand FasL are responsible for ALPS type 1a and ALPS type 1b, respectively. ALPS type 2 is a clinical variant caused by mutations in the caspase-10 gene. Fas is a key receptor in the apoptotic pathway and the binding of FasL to Fas leads to apoptosis by activating a series of events involving a group of proteases called caspases. The defective apoptotic function in ALPS leads to an accumulation of lymphocytes (particularly the 'double negative' T cells – DNT, $CD3^-CD4^-CD8^-$), including potentially autoreactive cells.

ALPS tends to follow a chronic course, with a variable response to immunosuppressive drugs such as methylprednisolone and in particular sirolimus and/or MMF. Splenectomy is indicated occasionally but these patients can also be neutropenic and there is a substantial increased risk of infections post-surgery. Supportive therapy with intravenous immunoglobulin replacement and long-term antibiotic prophylaxis is important in these patients. Long-term outcome is variable, although survival into adulthood is well recognized. There is an increased risk of malignancy (lymphoma in particular). Allogeneic bone marrow transplantation has been successful in some children. An expanding number of similar single gene disorders characterized by lymphoproliferation and autoimmune phenomena are now being described. The extent to which these disorders will be characterized by endocrine dysfunction is unclear [66].

Kabuki Make-Up Syndrome (KMS)

KMS is a syndrome characterized by five manifestations: (1) dysmorphic face with eversion of the lower lateral eyelid, arched eyebrows with sparseness of their lateral one-third, long palpebral fissures with long eyelashes, depressed nasal tip and prominent large ears (100%), (2) unusual dermatoglyphic patterns (96%), (3) skeletal abnormalities and hypermobile joints (88%), (4) mild to moderate mental retardation (84%), and (5) postnatal growth retardation with short stature (55%) [67].

Other well-recognized features include dental abnormalities, susceptibility to infections, particularly recurrent otitis media, cardiovascular anomalies, renal and urinary tract anomalies, biliary atresia, diaphragmatic hernia and anorectal anomalies. Less common associations include growth hormone deficiency, primary ovarian dysfunction, Hashimoto's thyroiditis, T1D and vitiligo [67]. Other endocrine abnormalities reported in these patients include isolated premature thelarche in around 25% of individuals as early as 4 months of age. Elevated gonadotropin concentrations, particularly FSH, are found and the aetiology has been postulated to be secondary to low hypothalamic sensitivity to the suppressive effects of sex hormones on gonadotropin secretion.

Rare endocrine findings include true central precocious puberty, growth hormone deficiency, hyperinsulinaemic hypoglycaemia, congenital hypothyroidism and T1D. Dominant mutations in the chromatin regulators lysine (K)-specific methyltransferase 2D (KMT2D) (also known as MLL2) [68] and lysine (K)-specific demethylase 6A (KDM6A) underlie the majority of cases with rarer causes including mutations in *RAP1A*.

KMS has been recognized most commonly within the Japanese population (incidence 1 : 32,000) but it is now diagnosed in all countries. Patients often survive with a good prognosis unless they have severe complications such as cardiovascular, hepatic or renal disease. The endocrinopathies should be treated along standard lines.

STAT1

Gain-of-function mutations in the transcription factor signal transducer and activator of transcription 1 (STAT1) are associated with mucocutaneous candidiasis [68, 69]. Enhanced signalling appears to impair the differentiation of Th17 cells, a particular type of T-helper cell involved in defence against fungal infections although patients are more susceptible to viral and bacterial infections as well. Patients can also have other features reminiscent of IPEX including enteritis, T1D, growth failure and autoimmune thyroid disease. Enamel defects and chronic lung disease are also reported [70]. Key components of management include IVIG, fluconazole prophylaxis and nutritional support; inhibitors of JAK/STAT signalling pathway and allogeneic HSCT are potential treatment options for severely affected patients with features of combined immunodeficiency.

STAT3

The transcription factor, signal transducer and activator of transcription 3 (STAT3), has a key role in many cellular processes. Germline gain-of-function mutations in STAT3 result in a disorder characterized by lymphadenopathy, immunodysregulation with autoimmune cytopenias, multi-organ autoimmunity (including lung, skin, gut, joints), polyendocrinopathy (diabetes and thyroid) and short stature. Patients are susceptible to infections and lymphoproliferation with hypogammaglobulinaemia, decreased natural killer, T-helper 17 and regulatory T cells [71, 72]. Mutations appear to result in secondary defects in STAT5 and STAT1 phosphorylation. In addition to supportive care, there have been favourable reports of modulating STAT3 activity with monoclonal antibody against the interleukin-6 receptor (IL-6R), as IL-6 signals through STAT3 and of allogeneic HSCT. This phenotype contrasts to that of loss-of-function mutations in STAT3, which are associated with the primary immune deficiency autosomal dominant hyper-IgE syndrome (Job syndrome).

NFKB2

NFKB2 (nuclear factor kappa B subunit 2) mutations result in anterior pituitary deficiency with variable immune deficiency (DAVID syndrome). This is characterized by hypopituitarism, immune dysfunction including hypogammaglobulinaemia and autoimmunity, with hypothyroidism a well-recognized component [73]. Patients have B-cell deficiency with infections such as bacterial pneumonia, human herpes viruses and *Giardia* from an early age and reduced number of Treg cells. Features include alopecia, vitiligo and ITP. The hypopituitarism appears not to be autoimmune in nature, in part because there are structural changes in hypothalamo-pituitary anatomy. Patients may have other anterior pituitary hormone deficiencies including GH, TSH and Gn deficiency.

This disorder is due to dominant mutations in the NFKB2 gene with mutations frequently lying near the C-terminus of the protein-coding region. The mutations appear to result in gain of function with an unprocessed NFKB2 repressor protein. There are reports of successful allogeneic HSCT in these patients although this treatment will not address pituitary hormone deficiencies.

CTLA-4

Mutations in CTLA-4 (otherwise known as CTLA-4 haploinsufficiency with autoimmune infiltration or CHAI) result in lack of restraint and dysregulation of T-cell responses. The consequence is lymphadenopathy and lymphocytic infiltration of multiple nonlymphoid organs together with autoimmune disorders that include T1D, autoimmune thyroid disease, arthritis and skin disorders such as psoriasis, uveitis and vitiligo. A number of approaches have been used to treat CHAI including abatacept, a CTLA-4–immunoglobulin fusion drug, which may mimic some of the actions of CTLA-4. Sirolimus that suppresses T-cell responses has also been used to alleviate autoimmune manifestations in these patients. There are a number of successful reports of HSCT.

LRBA Deficiency

Lipopolysaccharide-responsive and beige-like anchor protein (LRBA) mutations result in a syndrome with a varied phenotype that includes hypogammaglobulinaemia, autoimmunity, inflammatory bowel disease and lymphoproliferation. Additional reported clinical features include autoimmune hepatitis, autoimmune haemolytic anaemia, ITP and splenomegaly. T1D and autoimmune thyroid disease are among reported endocrinopathies [74, 75]. In addition to decreased IgG antibody production, there is deficient T-cell activation, proliferation and apoptosis with impaired cell-mediated suppression and decreased expression of Treg cell markers, such as CD25, FOXP-3 and CTLA4. LRBA is an important component in the immune system response to infections but also has an important role in cell proliferation, cell death and immune regulation. LRBA is a large intracellular protein that has been found to be involved in CTLA4 turnover by impacting on lysosomal degradation. LRBA appears to maintain intracellular stores of CTLA4, which can then mobilize quickly to the cell surface where it can, in turn, downregulate T-cell function. A broad range of immunosuppressive therapies have been used to treat patients with LRBA mutations including corticosteroids, immunoglobulin replacement, MMF, tacrolimus and infliximab. A favourable response to abatacept has also been reported in terms of susceptibility to autoimmune disease although patients remain

susceptible to infections [76]. Some patients have been treated successfully with HSCT.

Conclusion

Diagnosis and management of the polyglandular syndromes has many dimensions. In the modern era, we should aim to use the powerful combination of clinical skills, autoantibody assays and molecular genetic investigation, along with basal and dynamic endocrine testing, to institute early diagnosis and therapy for these conditions. In the future, more accurate disease prediction may allow us to counsel individuals and families with greater certainty. Ultimately, when pathogenesis is more clearly understood, specific intervention to prevent endocrinopathy in those at high risk may become more than a theoretical possibility.

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12

Endocrine Neoplasia

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KEY LEARNING POINTS

- Endocrine neoplasia in children is rare and its management is challenging.
- Children typically present with clinical features of hormone excess or enlargement of an endocrine gland but optimal management often requires collaboration between many professionals.
- Lifelong endocrine therapies or surveillance for recurrent or associated disease is required as well as close collaboration with specialists in adult practice, facilitating seamless transition into adult medical services.
- Genetic counselling and genetic testing are recommended for children with medullary thyroid carcinoma, paragangliomas and phaeochromocytomas and adrenocortical cancer.
- ^{131}I treatment is not recommended for children with low-risk papillary thyroid cancer.
- 40% of phaeochromocytomas are familial, 8–43% extra-adrenal and 7–53% bilateral or multifocal.
- The classic triad of symptoms in patients with a phaeochromocytoma consists of episodic headache, sweating and tachycardia.
- The virilizing syndrome is the most common presentation for adrenocortical cancer.
- Ovarian enlargement, whether cystic or solid, must be evaluated to exclude malignancy (10–20% malignancy rate).
- Cisplatin-based chemotherapy cures a substantial proportion of metastatic testicular germ cell tumours.

Endocrine neoplasia in children is rare and its management is challenging. Children typically present with clinical features of hormone excess or enlargement of an endocrine gland but optimal management often requires collaboration between a large number of professionals, including pediatric endocrinologists, oncologists and specialist surgeons, as well as experienced geneticists and pathologists. Furthermore, many children will require lifelong endocrine therapies or surveillance for recurrent or associated disease and close collaboration with specialists in adult practice facilitates seamless transition into adult medical services.

In this chapter we review the epidemiology, clinical presentation, diagnosis and management of the most common pediatric endocrine neoplastic diseases that physicians deal with in daily clinical practice. Since endocrine neoplasia is often associated with inherited genetic abnormalities, we review the most common hereditary syndromes that predispose to these conditions. We emphasize the need of national and international collaboration for the optimal management of these patients given the lack of data from randomized controlled trials due to the rarity of these tumours.

Thyroid Neoplasia: Nodules and Cancer

Approximately 2% of children have palpable thyroid nodules. Most are benign, including inflammatory lesions and follicular adenomas, but a few are malignant. Thyroid cancer is the most common pediatric endocrine malignancy and the third most common pediatric solid tumour, accounting for 1–1.5% of all childhood cancers with an incidence of 0.5 per million per year. Females are more commonly affected than males and the female to male ratio increases steadily with age.

Epidemiology

75% of thyroid nodules in children disappear with age. Palpation detects thyroid nodules in 1.8% of schoolchildren 11–18 years of age but, in a follow-up examination

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Companion website: www.wiley.com/go/dattani/brookcpe7

conducted 20 years later, nodules were detectable in only 0.45% of subjects. Thyroid nodules can be detected by contrast-enhanced computed tomography (CT), a study that is not preferred today given its exposure to irradiation, in 1.4% of children without suspected thyroid disease. Most are benign but 5.7% of the nodules detected incidentally by contrast-enhanced CT in the above-cited study were malignant. This high percentage probably represents 'referral bias' given that an analysis of the Surveillance, Epidemiology and End Results (SEER) registry from 1973 to 2004 revealed that approximately only 1 in 180 solitary nodules was thyroid cancer (0.5%) [1].

Pathogenesis

a) **Radiation** – Radiation is a well-recognized risk factor for thyroid cancer, the risk being strongly related to the cumulative dose. A history of external irradiation for treatment of childhood malignancies increases the risk of developing thyroid cancer and lifelong surveillance with annual ultrasound examination of the thyroid is strongly recommended. The latent period between exposure and the appearance of thyroid cancer may be up to 40 years.
b) **Genetic conditions** – Several genetic syndromes have been linked to an increased risk of thyroid cancer:

- *Gardner syndrome*, an autosomal dominant disorder caused by a mutation in the *APC* (adenomatous polyposis coli) gene, is associated with familial adenomatous polyps in the gastrointestinal tract and papillary thyroid cancer.
- *Cowden* and *Bannayan–Riley–Ruvalcaba* syndromes, both associated with germline mutations in the *PTEN* gene, are autosomal dominantly inherited PTEN hamartoma tumour syndromes and characterized by hamartomas in the skin and other tissues and an increased predisposition to thyroid cancer.
- *Carney complex* is a multiple neoplasia syndrome associated with a mutation in the protein kinase A regulatory subunit type 1 alpha gene (*PRKAR1A*). It is characterized by primary pigmented nodular adrenocortical disease (PPNAD), other endocrine tumours including papillary or follicular thyroid cancer (FTC) and non-endocrine tumours such as myxomas and breast adenomas.
- *Multiple endocrine neoplasia type 2 (MEN2)* is associated with medullary thyroid cancer and caused by germline mutations in the RET proto-oncogene. There are three distinct subtypes of MEN2, all associated with medullary thyroid carcinoma (MTC): MEN2A, MEN2B and familial medullary thyroid cancer (FMTC).
- Thyroid cancer in children has also been associated with Werner syndrome, germline DICER defects and *HABP-2* mutations (hyaluronan-binding protein 2, a tumour suppressor gene) as well as thyroglossal duct cysts, and other defects of normal thyroid development.

Clinical Presentation and Diagnostic Evaluation

83% of childhood thyroid cancers are papillary carcinomas, 10% are follicular, 5% are medullary carcinomas and 2% are 'other' types of thyroid cancer [2]. A painless mass is the commonest presentation of thyroid cancer, which is frequently an incidental finding on routine examination or identified by the child or parent.

Clinical findings that increase the likelihood of cancer are a history of radiation to the head and neck, a firm or fixed mass with rapid growth, enlarged cervical lymph nodes (LNs) and symptoms of hoarseness or dysphagia. Most children do not have these signs and symptoms that are common in adults with thyroid malignancy. Neither size nor thyroid hormonal status is predictive of malignancy but most malignant tumours are more than 1.5 mm in diameter and 80–90% of patients are euthyroid at presentation.

Thyroid hormone measurement, imaging by scintigraphy and ultrasound and fine needle aspiration (FNA) are commonly used to evaluate thyroid nodules and identify those that are malignant. Diagnostic evaluation is similar to that used in adults but nodules are more likely to be malignant in children.

Biochemical Evaluation

Thyroid hormone evaluation is essential in all children presenting with a thyroid nodule. Most thyroid function tests are normal and ultrasound neck imaging is the next step. For patients with low TSH concentrations, a hyperfunctioning nodule is the most common diagnosis and thyroid scintigraphy should be performed in addition to neck ultrasound.

Imaging

Neck ultrasound

Thyroid ultrasound should assess the thyroid nodules and evaluate the cervical LNs. The first question is whether there are one or multiple nodules and their size. Specificity of ultrasound for malignancy is not perfect but microcalcifications, abnormal margins, hypoechogenicity, lack of capsule and increased vascularity are all suggestive of malignancy. Cystic lesions are rarely malignant.

Thyroid Scintigraphy

This gives information on iodine uptake. If serum TSH is low (suggesting a hyperfunctioning or 'hot' nodule),

thyroid scintigraphy is usually performed by using ^{123}I as a tracer. Most nodules show reduced uptake; those with increased uptake are rarely malignant. Scintigraphy has relatively low sensitivity and fails to identify ~20% of the nodules found by ultrasound; it is used mainly for the evaluation of thyrotoxic patients and referral of patients with an autonomous nodule for surgery.

Fine needle Aspiration (FNA)

FNA is the gold standard for separating benign from malignant nodules and referral of patients for thyroidectomy. The diagnostic accuracy of FNA in childhood varies from 77–90.4% with 89–100% sensitivity and 63–83% specificity [1]. When an experienced team performs the procedure, complications (e.g. haemorrhage, fibrosis or abscess formation) are rare. Selection of patients for FNA is usually based on clinical features and nodule characteristics on ultrasound. FNA biopsy is recommended for nodules with the following characteristics:

- Larger than 1 cm with solid component (pure solid or mixed solid/cystic),
- Smaller than 1.0 cm, if they have microcalcifications or abnormal (enlarged) regional cervical LNs [3].
- Documented progressive enlargement on repeat ultrasound examinations.

Genetic Testing

Genetic counselling and testing are recommended for children with findings on physical examination, endoscopy and family history. Children with non-medullary thyroid cancer should be especially evaluated for clinical features of a PTEN hamartoma syndrome, which include macrocephaly, developmental delay, lipomas and other benign skin lesions. Children with any of these features, particularly macrocephaly, should be tested for a PTEN mutation. If gastrointestinal polyps are present, familial adenomatous polyposis should be considered and *APC* genetic testing is recommended. *RET* proto-oncogene analysis is strongly recommended in MTC (see below).

Management

Benign Thyroid Nodules

Watchful waiting with periodic neck palpation and ultrasound is warranted for FNA-diagnosed benign nodules. An increase in size merits repeat FNA or surgical excision (usually a lobectomy), given that cancer may be present in a small percentage of these 'benign nodules'. Immediate repeat FNA is a reasonable option when the cytology specimen is not diagnostic or suggestive of atypia or of a lesion of undetermined significance. Surgery is reserved for follicular neoplasms that are associated with a significant cancer risk (up to 25%).

Papillary Thyroid Cancer

Children tend to have multifocal disease and a high risk of tumour recurrence, either locally or nearby in cervical LNs. Given these observations, the procedure of choice is total or near-total thyroidectomy and cervical LN dissection. It is important for these children to be treated at centres with high volume and experienced surgeons.

The AJCC TNM classification system (Table 12.1), widely used for describing the extent of disease and prognosis in the adult population, is excellent for describing the extent of disease and stratifying an approach to evaluation and management of children. Using the TNM classification system, especially regional LN and distant metastasis staging, the American Thyroid Association (ATA) categorizes pediatric patients into one of three risk groups [4]:

Table 12.1 Thyroid cancer TNM staging.

Primary tumour (T)		
TX		Size not assessed, limited to the thyroid
T1	T1a	≤1 cm, limited to the thyroid
	T1b	>1 cm but ≤2 cm, limited to the thyroid
T2		>2 cm but ≤4 cm, limited to the thyroid
T3		>4 cm, limited to the thyroid or any tumour with minimal extrathyroidal extension
T4	T4a	Tumour extends beyond the thyroid capsule to invade subcutaneous soft tissues, larynx, trachea, oesophagus or recurrent laryngeal nerve
	T4b	Tumour invades prevertebral fascia or encases carotid artery or mediastinal vessels
Lymph nodes (N)		
NX		Regional lymph nodes not assessed
N0		No regional lymph node metastasis
N1	N1a	Metastasis to level VI (pretracheal, paratracheal and prelaryngeal/Delphian lymph nodes)
	N1b	Metastasis to unilateral, bilateral or contralateral cervical levels I, II, III, IV or V or retropharyngeal or superior mediastinal lymph nodes (level VII)
Distant metastasis (M)		
MX		Distant metastasis not assessed
M0		No distant metastasis
M1		Distant metastasis

Source: AJCC Cancer Staging Manual, 7th edition (2010) published by Springer Science and Business Media LLC. 2010 Thyroid.
Pediatric patients are considered to have stage II disease if distant metastases are identified (M1); otherwise, all pediatric patients are considered to have stage I disease.

Low risk: Disease grossly confined to the thyroid with N0 or NX disease or patients with incidental N1a metastasis in which 'incidental' is defined as the presence of microscopic metastasis in a small number of central neck LNs. These patients appear to be at lowest risk for distant metastasis but may still be at risk for residual cervical disease, especially if the initial surgery did not include cervical node dissection.

Intermediate risk: Extensive N1a or minimal N1b disease. These patients appear to be at low risk for distant metastasis but are at an increased risk for incomplete LN resection and persistent cervical disease.

High risk: Regionally extensive disease (extensive N1b) or locally invasive disease (T4 tumours), with or without distant metastasis. Patients in this group are at the highest risk for incomplete resection, persistent disease and distant metastasis.

For intermediate- and high-risk patients, a post-operative ^{123}I scan and a serum thyroglobulin concentration (measured while on thyroid hormone treatment for low-risk or TSH-stimulated for intermediate- and high-risk patients) is indicated. If serum thyroglobulin levels are elevated (usually >2 ng/mL), the patient possibly has microscopic residual disease. If an ^{123}I scan shows uptake outside the thyroid bed, further imaging is warranted to detect the abnormal LN(s). Meticulous ultrasound examination is the first step but, if it fails to identify residual disease, imaging with contrast-enhanced CT or magnetic resonance imaging (MRI) are reasonable options. If the residual disease is resectable (e.g. abnormal LNs), surgical excision is the best approach; radioiodine treatment is reserved for children with widespread distant metastases or local disease not amenable to surgery.

^{131}I treatment is not recommended for children classified as low risk, i.e. cancer confined to the thyroid gland with no or only microscopic metastases in a small number of central neck LNs and with a serum thyroglobulin <2 ng/mL [4]. Such treatment is reserved for children at intermediate or high risk to attempt to eradicate residual disease. Adjuvant radioiodine treatment has been associated less often for local recurrence. If radioactive iodine administration is indicated, either for diagnosis or treatment, elevated TSH concentrations are needed. Thyroid hormone withdrawal and recombinant human thyrotropin (TSH) administration have been shown to be equally effective in achieving these [5].

Thyroxine replacement should start 3 days after the administration of ^{131}I. The aim should be to maintain the child in a clinically euthyroid state with serum T4 and T3 in the near-normal range while suppressing TSH. ATA guidelines recommend a TSH goal depending on DTC risk level: low-risk goal 0.5–1.0 mIU/L, intermediate-risk goal 0.1–0.5 mIU/L and high-risk goal <0.1 mIU/L [4].

Although patients remain at lifelong risk for recurrence, it occurs most commonly in the first 10 years following surgery. Therefore, surveillance after treatment is indicated for all children treated for thyroid cancer to detect recurrent disease as early as possible as well as diagnose and manage complications of supraphysiological doses of thyroxine and ^{131}I late toxicities (e.g. pulmonary fibrosis, lymphadenitis pulmonitis, chronic sialadenitis, bone marrow suppression, leukaemia and ovarian damage). History and physical examination for thyroid masses and cervical lymphadenopathy can be augmented by serum Tg, neck ultrasound and ^{131}I or ^{123}I diagnostic whole body scan. Close collaboration between endocrinologists, thyroid surgeons and nuclear medicine physicians is essential and should be maintained after the transition from pediatric care to adult services. Optimal care requires careful planning, excellent communication between clinicians and patients and, wherever possible, a period of joint consultation during adolescence.

Recurrent disease should be confirmed by biopsy and surgical excision is the treatment of choice for palpable lesions and bulky lesions affecting bone and some mediastinal lesions if possible. If lesions are not amenable to surgery, ^{131}I in a dose of 100–270 Ci should be administered 6-monthly.

Follicular Thyroid Cancer (FTC)

The clinical behaviour of FTC in children is different from PTC in that it may be less aggressive and is generally associated with less advanced disease, fewer distant metastases and a lower rate of recurrence. Except for the aggressive variants, FTC is typically unifocal, rarely spreads to regional LNs and may have autonomous function but it is prone to early haematogenous metastases, even in the absence of cervical node involvement. It has an excellent prognosis [2]. In children diagnosed with FTC, consideration should be given to genetic counselling and testing for germline *PTEN* mutations, particularly in a child with macrocephaly or with a family history suggestive of the *PTEN* hamartoma tumour syndrome.

Depending on the extent of invasion, FTC divides into minimally invasive and widely invasive forms. Tumours with microscopic capsular invasion alone and/or very limited vascular invasion are classified as minimally invasive, whereas neoplasms that show widespread infiltration into blood vessels and/or adjacent thyroid tissue and often lack complete tumour encapsulation are deemed widely invasive. Patients with evidence of vascular invasion (more than three involved blood vessels), known distant metastasis and/or tumour size >4 cm should undergo total thyroidectomy with ^{131}I post-operatively. Minimally invasive FTC <4 cm in size and with no or minimal vascular invasion (three or fewer involved blood vessels)

should be treated on a case-by-case basis but lobectomy with ^{131}I therapy may be sufficient [4].

Paragangliomas and Phaeochromocytomas

Phaeochromocytomas (PHEOs) and paragangliomas (PGLs) are rare neuroendocrine tumours (NETs) arising from neural crest-derived cells or organs known as paraganglia. In 2004, the World Health Organization (WHO) defined PHEO as an intra-adrenal PGL, whereas tumours of extra-adrenal sympathetic or parasympathetic paraganglia were classified as extra-adrenal PGLs [6]. The medulla and the organ of Zuckerkandl are the prototypical sympathetic paraganglia. Parasympathetic paraganglia are found mainly along the supradiaphragmatic branches of the vagus and glossopharyngeal nerves. The prototypical parasympathetic paraganglion is the carotid body. Sympathetic PHEOs/PGLs usually secrete catecholamines (chromaffin tumours). Parasympathetic PGLs were thought not to secrete catecholamines until relatively recently, when studies found that as many as 20% of head and neck PGLs also produce significant amounts of catecholamines. PGLs are located anywhere from the base of the skull to the pelvis. In general, ~80% of all these tumours are located in the adrenal medulla (PHEOs), whereas extra-adrenal PGLs most commonly arise from the head and neck or in the abdomen around the inferior mesenteric artery (organ of Zuckerkandl) and aortic bifurcation. Less commonly, they are in the prevertebral region, in the pelvis or the thorax.

Epidemiology

The annual incidence for PHEOs is 3–8 cases per 1 million per year in the general population. Approximately 10–20% of cases are diagnosed during childhood at an average age of 11 years, with a slight predominance in boys, particularly under the age of 10. In children, 40% of PHEOs are familial, 8–43% extra-adrenal and 7–53% bilateral or multifocal [7].

Pathology

The term phaeochromocytoma is accredited to the pathologist Ludwig Pick, who noted the dark brown colour of the tumour cells when in contact with fixatives containing chromic acid salts ('phaeo' is the Greek word that describes a brown or dusky appearance). The gross and microscopic appearances of PHEOs and PGLs are characteristic: the tumour consists of large polygonal epithelioid cells arranged in a nested (zellballen) pattern with an investiture (often discontinuous) of spindled cells. The stroma around the nests of cells is characteristically endowed with a rich vascular component. Nevertheless, the classic zellballen pattern is often not evident and one may instead observe diffuse architecture, spindle cells, admixtures of large and small cells and extreme cytologic atypia.

The single most specific and reliable neuroendocrine marker currently used in pathology practice is chromogranin A (CgA), a major constituent of catecholamine-containing secretory granules. Immunoreactivity for CgA will readily distinguish PHEOs and other PGLs from tumours that are not neuroendocrine, such as those of the adrenal cortex. There is also immunopositivity for synaptophysin and neurofilament, while S100 staining is found at the circumferential sustentacular cells. Recently, tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine biosynthesis, has been added to the panel of markers that can be used to distinguish PHEOs and PGLs from other NETs that also express CgA. Staining for CgA and TH may be weaker and more variable in parasympathetic PGLs than in their sympathoadrenal counterparts.

It is difficult, if not impossible, to distinguish malignant from benign PHEOs based on histopathological features. The phaeochromocytoma of the adrenal gland scaled score (PASS) was developed to distinguish benign from malignant PHEOs. A PASS of ≥4 is associated with a higher probability for malignancy. Immunohistochemistry has been used in addition for assessment of malignant potential. Proliferation marker Ki-67 (tested with monoclonal antibody MIB-1) appears to correlate well with malignancy in PHEOs; Kimura et al. [8] proposed a scoring system that adds up to a maximum of 10 and includes Ki-67 immunoreactivity along with catecholamine and phenotype. With a score of 7–10, 100% of patients were found to have malignant tumours.

Pathogenesis

PHEOs/PGLs may occur sporadically or as part of a hereditary syndrome. The latest studies have indicated that ~25% of apparently sporadic or non-syndromic PHEOs or PGLs may be hereditary.

One of the reasons may be that low-penetrance molecular variants in key genes may cause sporadic PHEOs or PGLs, at least in some cases, by low oxygen concentrations in the tidal air. The tissues where these tumours arise from (e.g. glomus cells of the carotid body, neuroepithelial bodies in the lungs, chromaffin cells of the fetal adrenal medulla and vascular smooth muscle cells) sense local oxygen tension. Evidence supporting this hypothesis was provided by anatomical changes in the

carotid body of high altitude dwellers in Peru exposed to chronic hypoxia whose carotid bodies were found to be substantially larger than those in sea-level living subjects. In 2000, a direct link between the hypoxic pathway and the emergence of PHEOs/PGLs was revealed with the identification of mutations in genes encoding for the succinate dehydrogenase (SDH) enzyme subunits A, B, C and D (*SDHA*, *SDHB*, *SDHC*, *SDHD*), collectively known as *SDHx*, or their cofactors [9].

Genetic Syndromes Associated with PHEOs/PGLs

Syndromic occurrence of PHEOs/PGLs is found in multiple endocrine neoplasia 2 (MEN2A or MEN2B), neurofibromatosis type 1 (NF1), von Hippel–Lindau (VHL) syndrome and familial PGLs due to germline mutations of genes encoding the four subunits of SDHx (SDHA, SDHB, SDHC and SDHD) of mitochondrial complex II. PGLs have also been found in the context of rare genetic conditions that predispose to a variety of hamartomas or sarcomas, such as Carney triad and Carney–Stratakis syndrome (Table 12.2). The main syndromes associated with PHEOs/PGLs will be discussed here.

Multiple Endocrine Neoplasia Type 2 (MEN2)

MEN2 is an autosomal dominant syndrome caused by activating germline mutations in the *RET* proto-oncogene located on chromosomal location 10q11.2, which encodes a transmembrane tyrosine kinase receptor for ligands belonging to the glial cell line-derived neurotrophic factor family of proteins involved in regulation of cell proliferation and apoptosis [11]. The MEN2 syndromes include MEN2A, MEN2B and FMTC. In each subtype, MTC is preceded by C-cell hyperplasia, which progresses to a multicentric neoplasm. Patients with MEN2A and MEN2B also develop PHEOs but almost never PGLs. Parathyroid hyperplasia/adenomas occur in MEN2A but are rare in MEN2B. MEN2B is also characterized by multiple mucosal ganglioneuromas and a skeletal condition in which most patients have a Marfanoid habitus. In most cases, especially within the context of MEN2B, MTC is the first presentation of MEN2.

Pathogenic gain-of-function germline *RET* mutations are found in over 95% of patients with the MEN2A or MEN2B clinical phenotypes and in ~85% of individuals with FMTC. Nearly all RET mutations in patients with MEN2A involve missense mutations that replace one of the six extracellular cysteine residues close to the transmembrane domain of the RET protein. Codon 634 mutations, particularly p.C634R, are highly associated with MEN2A [12].

While there is a clear genotype–phenotype association in MEN2, the correlations are imperfect. For example, an identical germline *RET* mutation may produce FMTC in one family and MEN2A in another. The basis for this is unclear but may be due to the influence of additional genetic modifiers within the *RET* gene itself as well as in other genetic loci. >98% of the MEN2B phenotype appears to be produced by two specific missense mutations in the tyrosine kinase domain of the *RET* proto-oncogene, p.M918T (>95% of MEN2B) and p.A883F (2–3%) [13].

Identification of a *RET* gene mutation within a MEN2 family is of great benefit for those affected because MTC can be prevented or cured by early prophylactic thyroidectomy and biochemical monitoring for PHEO allows its early detection and therapy. It is obvious that any family with hereditary MTC with or without PHEO needs testing. Individuals with apparently sporadic MTC should also be tested because the prevalence of germline RET mutations in this group of patients with MTC but no apparent family history is ~5–10%.

PHEOs associated with MEN2 are usually diagnosed in the third to fourth decades of life and are frequently benign and bilateral in more than 50% of cases [11, 14]. Malignant PHEO is rare but children with MEN2B-associated PHEOs have a higher risk of malignancy compared with those with MEN2A or sporadic disease [14]. Any child at risk for MEN2 who develops signs and symptoms of PHEO, such as headache, irritability or hypertension, should be evaluated for the presence of the tumour. Annual screening through measurement of fractionated metanephrines (urine or plasma) is indicated, even though some patients with PHEO present with radiographic abnormalities before they are detected with increased excretion of catecholamines.

This is probably a reflection of the adrenal medullary hyperplasia that is a precursor of PHEO in the context of MEN2 syndromes [14, 15]. MEN2-related PHEOs overexpress phenylethanolamine *N*-methyltransferase (PNMT), the enzyme that converts norepinephrine to epinephrine. In these cases, large amounts of epinephrine and its catechol-O-methyltransferase (COMT) metabolite metanephrine are detected, which can be useful in the differential diagnosis between MEN2 syndromes and the catecholamine-secreting tumours in the context of VHL and the familial PGL syndromes [11].

Neurofibromatosis Type 1 (NF1)

NF1 is a disease inherited in an autosomal dominant fashion with an almost complete penetrance but variable expression. It is caused by inactivating mutations or deletions in the *NF1* gene localized at 17q11.2 that acts as a tumour suppressor.

Disease incidence is ~1 in 2600 to 1 in 3000 individuals. Approximately half of the cases are familial, while the remaining ones represent *de novo* mutations.

Table 12.2 Familial syndromes presenting with PHEOs/PGLs and their characteristics.

Syndrome	Gene/locus	Mode of inheritance	Clinical presentation	Particular characteristics
MEN2	***RET*** – 10q11.2	Autosomal dominant	• MEN2A: MTC, hyperparathyroidism, PHEO • MEN2B: MTC, PHEO, Marfanoid habitus, mucosal ganglioneuromas	• Diagnosis: 3rd–4th decade • Benign, bilateral (50%) • Almost exclusively secrete metanephrine
NF1	***NF1*** – 17q11.2.	Autosomal dominant	• Café au lait spots, intertriginous freckling, Lisch nodules, neurofibromas, optic nerve gliomas, dysplasia of sphenoid bone, PHEO	• Diagnosis: 5th decade • Unilateral • Produce epinephrine, norepinephrine
VHL	***VHL*** – 3p25-26	Autosomal dominant	• CNS haemangioblastomas; retinal angiomas; renal cell carcinomas; cysts of the kidney, pancreas, epididymis and liver; haemangiomas of the adrenal, liver and lungs; PHEO (type II); rarely PGLs	• Bilateral (50%) • Almost exclusively secrete norepinephrine • Malignancy rate: <7%
PGL1	***SDHD*** – 11q23	Autosomal dominant	• Parasympathetic head/neck PGLs, sympathetic PGL (rare), unilateral/bilateral PHEO (rare)	• Diagnosis: 35 years (mean) • Secrete norepinephrine, dopamine • Risk of malignancy: low • Maternal imprinting
PGL2	***SDH5*** – 11q13.1. (*SDHAF2*)	Autosomal dominant	• Head/neck parasympathetic PGLs; no PHEOs yet	• Age at diagnosis: 33 years • Rate of malignancy: unknown • Maternal imprinting
PGL3	***SDHC*** – 1q21	Autosomal dominant	• Head/neck PGLs, sympathetic PGLs and PHEOs	• Mean age of onset: 50 years • Risk of malignancy: very rare • No influence by the parent of origin
PGL4	***SDHB*** – 1p35–p36	Autosomal dominant	• Sympathetic and parasympathetic PGLs/PHEOs; increased risk for renal cell carcinoma and breast and papillary thyroid carcinoma	• Mean age of diagnosis: 30 years • Malignancy rate: 34–70% • Secrete norepinephrine, epinephrine, dopamine, methoxytyramine
Carney triad	Unknown – 1p, 1q deletions Other	Unknown	• Pulmonary chondroma, GISTs, functioning PGLs/PHEOs. Other tumours: adrenocortical adenoma, oesophageal, duodenal and pancreatic islet cell tumours	• Mean age of diagnosis: 25 years • Sympathetic and parasympathetic PGLs • PHEOs (mostly unilateral) • Rate of malignancy: 10%
Carney–Stratakis syndrome	***SDHB***, ***SDHC*** and ***SDHD***	Autosomal dominant	PGLs/GISTs	Multifocal, functioning and non-functioning PGLs

Source: Adopted by Xekouki and Stratakis [10].
CNS, central nervous system; GIST, gastrointestinal stromal tumour; MEN, multiple endocrine neoplasia; MTC, medullary thyroid carcinoma; NF1, neurofibromatosis 1; PGL, paraganglioma; PHEO, phaeochromocytoma; VHL, von Hippel–Lindau.

Most new mutations occur in paternally derived chromosomes. Neurofibromin is a cytoplasmic protein 2818 amino acids long that acts as a cell growth inhibitor. Analysis of the NF1 predicted sequence of neurofibromin revealed that it probably functions as a negative regulator of RAS, a key intracellular signalling protein important for regulating cell growth and survival, by accelerating the conversion of Ras-GTP into Ras-GDP. Loss of neurofibromin results in unopposed Ras activity leading to activation of several important downstream

signalling intermediates, including the mammalian target of rapamycin (mTOR) protein, and increased cell growth.

The clinical diagnosis of NF1 is based on criteria developed at a consensus conference in 1987 and updated in 1997. Any two of the following clinical features should be present: (1) café au lait spots, (2) intertriginous freckling, (3) Lisch nodules (benign iris hamartomas), (4) neurofibromas, (5) optic pathway gliomas, (6) distinctive bony lesions and (7) a first-degree family relative with NF1.

Other tumours may also occur more commonly in NF1, including gastrointestinal tumours, MTC, malignant gliomas, juvenile chronic myeloid leukaemias and malignant peripheral nerve sheath tumours. PHEOs are found in <1–2% of patients with NF1. In most of the cases (90%), they are benign and typically occur in the adult population. Unlike the other syndromes, NF1-associated PHEOs seem to share many features with sporadic PHEOs, including an older mean age (diagnosis is in the fifth decade), rare extra-adrenal location, unilateral presentation and a similar frequency of malignancy compared with the sporadic PHEO (12%). NF1-related PHEOs usually produce both epinephrine and norepinephrine. Because of the low incidence of PHEOs in the setting of NF1, routine screening of NF1 patients is not generally recommended but, if a patient with NF1 develops hypertension or any other symptoms suggestive of catecholamine excess, PHEO should be excluded.

von Hippel-Lindau Disease (VHL)

von Hippel–Lindau disease is inherited in an autosomal dominant manner with an incidence of 1 in 36,000 live births and a penetrance of 97% by age 60 years. The *VHL* gene is located at chromosome 3 (3p25-26) and functions as a tumour suppressor gene. As in NF1 and according to Knudson's 2-hit hypothesis, an inherited germline mutation of *VHL* and loss of function of the wild-type allele are needed for disease development.

The initial manifestations of disease can occur in childhood, adolescence or adulthood, with a mean age at initial presentation of about 26 years. The spectrum of VHL-associated tumours includes haemangioblastomas of the brain (cerebellum) and spine; retinal angiomas; clear cell renal carcinomas (RCCs); PHEOs; endolymphatic sac tumours of the middle ear; cysts of the kidney, pancreas, epididymis and liver; and haemangiomas of the adrenal gland, liver and lungs.

A distinct genotype–phenotype correlation exists, especially in the development of PHEOs. Patients in kindreds with type I disease have a substantially lower risk of developing PHEOs, although they are at high risk for the other VHL-associated lesions. Kindreds with type II disease are at high risk for developing PHEO.

Type II disease is subdivided by the risk of developing RCC. Type IIA and IIB families have a low and high incidence of RCC, while type IIC kindreds are characterized by the development of PHEOs only, without RCC or haemangioblastoma. Extra-adrenal sympathetic PGLs and parasympathetic head and neck PGLs can be found but they are infrequent. Less than 30% of patients with a *VHL* germline mutation develop a PHEO. These tumours, mainly located in the adrenal gland, are bilateral in about 50% of cases and undergo malignant transformation less frequently than sporadic PHEOs (<7%). As with MEN2, VHL PHEOs arise on the background of adrenomedullary hyperplasia and up to 40% of young populations with apparently sporadic PHEOs are carriers of a germline mutation in one of the genes responsible for the inherited forms of PHEO; VHL is the most frequent hereditary disease in this group of patients.

Hypertension is the most common symptom of PHEOs in VHL patients followed by headache and sweating; about 30% of patients with VHL can be normotensive and asymptomatic without evidence of increased catecholamine production. Among children undergoing genetic screening because of a familial disease, tumours can be detected in patients with or without high blood pressure. PHEOs from VHL patients produce only norepinephrine in 98% of cases due to low expression of PNMT.

There has been significant progress in the past few years on the physiological role of the VHL gene. The VHL protein is an E3 ubiquitin ligase that targets substrates for proteasome degradation. The best-known VHL substrates are the transcription factor hypoxia-inducible factor 1 (HIF1) and its three alpha subunits, HIF1α, HIF2α and HIF3α. Only hydroxylated HIF1 can be targeted by VHL for degradation. This post-translational modification is carried out at two specific prolyl residues by a family of oxygen-dependent enzymes known as prolyl hydroxylases (PHDs) 1, 2 and 3 (also known as Egln2, Egln1 and Egln3). Under hypoxic conditions, non-hydroxylated HIF1α and HIF2α translocate to the nucleus, where they dimerize with HIF1β and bind to specific promoter elements of target genes.

In mutant VHL target tissues, HIF activity is enhanced due to reduced degradation in the absence of a functioning VHL, which leads to transcriptional activation of several HIF target genes, including critical angiogenic factors such as vascular endothelial growth factor (VEGF), enzymes involved in glucose metabolism and cell survival and many others. The activation of HIF1 by the inhibition of PHDs or VHL provides part of the molecular explanation for what has been called pseudohypoxia, a phenomenon first described by Otto Warburg in the 1920s; the Warburg effect describes the striking rate of glycolysis and lactate production in the presence

of normal oxygen concentrations in tumour cells. Warburg proposed that this might be related to a defect in mitochondrial respiration or some other mechanism that allows the tumour cell to function as hypoxic under normoxic conditions. The Warburg effect has been confirmed in a broad range of tumours and is the basis for the use of functional imaging strategies such as the [18F]-deoxyglucose positron emission tomography ([18F]-FDG PET) for the diagnosis of PHEOs/PGLs.

Familial PGLs

Familial PGLs (hereditary PGL syndromes) are inherited as an autosomal dominant trait. They are due to mutations in genes encoding for components of mitochondrial complex II (SDH) of the respiratory chain [9]. SDH is the rate-limiting step between the tricarboxylic acid cycle and the respiratory chain and catalyses the oxidation of succinate to fumarate and transfers electrons directly to the ubiquinone pool. It consists of four subunits, two hydrophilic (a flavoprotein [SDHA] and an iron–sulphur protein [SDHB]) and two hydrophobic membrane anchor subunits, SDHC and SDHD. SDHA serves as a substrate-binding site for succinate and together with SDHB forms the catalytic part of the enzyme. SDHC and SDHD serve as the membrane anchors and an ubiquinone site.

The discovery that mutations in genes coding for the subunits SDHB, SDHC and SDHD were associated with the formation of parasympathetic and sympathetic PGLs and PHEOs that could coexist [9] led to the unravelling of the axiom of the 10-rule proposed by Bravo in 1984. Until that time, it was suggested that 10% of PHEOs were bilateral, 10% malignant, 10% normotensive, 10% extra-adrenal and 10% of genetic origin. In 2002, Neumann et al. [16] reported that 25% of apparently sporadic PHEO cases had a germline mutation. This percentage may be as high as 40% or even higher in children and young adults. Four PGL loci have been identified and familial PGLs have been classified as PGL1, PGL2, PGL3 and PGL4 syndromes.

PGL1 is caused by inactivating mutations in the SDHD gene located on chromosome 11q23 and accounts for 50% of cases of hereditary PHEOs/PGLs. It is characterized primarily by parasympathetic PGLs of the head and neck, less frequently by sympathetic PGLs and rarely by unilateral or bilateral PHEOs. The mean age of diagnosis is about 35 years, with a 68% penetrance by age 40. Risk for malignant transformation in PGL1 is generally low. Mutation analysis has revealed missense, nonsense and large deletions. A genotype–phenotype correlation has been reported, with nonsense and splicing mutations being associated with earlier disease development and the presence of PHEO in addition to head and neck PGLs. Increased concentrations of norepinephrine, dopamine or both in combination are usually detected. Thus, increased levels of methoxytyramine (a metabolite of dopamine) are a valuable biochemical marker in this subset of tumours.

When the first kindred with PGL1 syndrome was reported, it became obvious that high penetrance depended on paternal transmission. This suggested maternal imprinting of SDHD. It remains unclear whether this is the case, because there is biallelic expression of SDHD in various tissues, the region (11q23) where *SDHD* is located is not genetically imprinted, the *SDHD* promoter has not been found methylated in neither normal adrenal medulla nor in PHEOs and, if the maternal allele was completely inactivated, tumours should arise in the absence of loss of heterozygosity (LOH), which is not the case. Hensen et al. [17] postulated that other imprinted genes, especially H19, are inactivated in the imprinted region of the short arm of chromosome 11 (11p15), the function of which is the negative control of insulin-like growth factor 2 (IGF2) expression. This hypothetical tumour suppressor would be active if maternally derived and inactive if paternally derived. In cases of complete or partial deletion of the maternal copy of chromosome 11, the active H19 is lost, which leads to overexpression of the paternal copy of IGF2 and tumour formation.

Müller [18] argued against this hypothesis and proposed another model. He suggested that there is a partial inactivation of the maternally derived *SDHD* by an unknown mechanism. Some residual activity of SDH is present in cells with a paternally derived mutation, which is adequate for the normal function of paraganglia cells. Nevertheless, chronic dysfunction of SDHD leads to the accumulation of reactive oxygen species (ROS), HIF1 and succinate. If this chronic cellular hypoxia is above a critical threshold, non-disjunction and partial or total loss of maternal chromosome 11 occur. This series of events may eventually lead to tumour formation.

PGL2 syndrome is not as prevalent as PGL1 but it shares the same parental pattern of transmission, implying a mechanism like that proposed for PGL1. It is characterized by parasympathetic PGLs of the head and neck only; no cases of PHEOs have been described yet. It was not until recently that this syndrome was identified to be due to heterozygous loss-of-function mutations in the SDH5 gene, now known as *SDHAF2*, which encodes for SDH assembly factor 2, which has a crucial role in SDH function. This gene is located on chromosome 11q13.1. The loss of *SDHAF2* results in the loss of SDH function and a reduction in the stability of the SDH complex, leading to reduced levels of all subunits. The average age at onset is 33 years (range of 16–80 years). The growth rate of PGLs is low, with a median increase of 1 mm/year and a doubling time of 4.2 years. The rate of malignancy, if any, is still unknown.

PGL3 is also inherited in an autosomal dominant manner due to loss-of-function mutations of the *SDHC* gene located on chromosome 1q21. SDHC encodes for the large (cybL) subunit of cytochrome b of complex II and, when mutated, it leads to formation of head and neck PGLs as well as sympathetic PGLs and PHEOs. The age of onset ranges from 17 to 70 years, with a mean age similar to that of sporadic disease (about 50 years of age). In general, the clinical behaviour of SDHC-related PGLs appears to be like that of benign sporadic PGLs. However, a malignant catecholamine-producing PGL at the carotid bifurcation has been reported in a patient with a splicing mutation. The parent of origin does not influence SDHC expression.

Finally, PGL4 also has an autosomal dominant pattern of inheritance and is caused by mutations in *SDHB* gene, which encodes the iron–sulphur subunit of SDH located on chromosome 1p35-p36. It is characterized by sympathetic PGLs (organ of Zuckerkandl, mediastinum, pelvis), PHEOs and parasympathetic head and neck PGLs. An increased risk for renal cell carcinoma, breast carcinoma and papillary thyroid carcinoma has also been detected. The age of onset ranges from 6 to 77 years, with a mean age of diagnosis of 30 years. Penetrance is a matter of debate, ranging from 35 to 75%.

PGLs in this disease subset are often large and solitary, with a high risk of metastatic spread (malignancy rates usually vary between 34 and 70%). It has been estimated that the risk of malignancy is >30% by age 70 years. Based on these observations and even if there is no previous history of familial disease, all patients with metastatic PGLs should be screened for *SDHB* mutations. Most of the SDHB-related PGLs over-secrete catecholamines. Nevertheless, 10% of these tumours can be biochemically silent or produce dopamine. Thus, like PGL1, increased levels of methoxytyramine could differentiate patients with *SDHB* mutations from those with *VHL*, *RET* and *NF1* mutations. Recently, staining for SDHB of PHEO and PGL tumours was demonstrated to be a cost-effective approach in the diagnosis of SDH-related PHEOs/PGLs. The authors performed immunohistochemistry staining for SDHB on 220 tumours. SDHB protein expression was absent in all 102 PHEOs and PGLs with an *SDHB*, *SDHC* or *SDHD* mutation but was present in all 65 paraganglionic tumours related to MEN2, VHL disease and NF1. Completely absent staining for SDHB was found in tumours with *SDHB* mutation, whereas weak diffuse staining often occurred with *SDHD* mutations [19].

Until recently, mutations in *SDHA* had never been described in hereditary PHEOs/PGLs. Biallelic SDHA mutations were shown to cause an early-onset encephalopathy known as Leigh syndrome. In 2010, Burnichon et al. [20] described a patient with an extra-adrenal PGL resulting from a loss-of-function germline mutation in *SDHA*. The authors showed LOH at the *SDHA* locus in the tumour and that *SDHA* mutation does lead to HIF stabilization and the subsequent activation of the hypoxic pathway. This mutation was present in 4.5% of a large series of PHEOs/PGLs.

Following this publication, mutations in *SDHA* were detected in 3% of apparently sporadic parasympathetic PGLs and PHEOs. The molecular mechanism responsible for the PGL formation is like that described for the VHL disease. It was shown that during HIF hydroxylation, α-ketoglutarate (2-OG) undergoes oxidative decarboxylation to generate succinate. In *SDH* mutant cells, succinate accumulates, leading to inactivation of PHD activity. SDH dysfunction may also lead to the generation of ROS. It has been shown that succinate can induce ROS production and that ROS themselves lead directly to the stabilization of HIF1.

Clinical Presentation

The diagnosis of a PHEO/PGL is usually suggested by the history in a symptomatic patient, by discovery of an incidental adrenal/extra-adrenal mass or from the family history in a patient with familial disease. The classic triad of symptoms in patients with a PHEO consists of episodic headache, sweating and tachycardia. Blood pressure may be elevated, while excessive sweating, visual disturbance, weight loss, nausea, vomiting, polyuria and polydipsia are common in children and can cause dehydration [7]. PHEOs account for only 1–2% of hypertension cases in children, so other more common causes such as renal disease and renal artery stenosis should be excluded [7].

Diagnosis

Advances in the assays used to detect increased catecholamine concentrations and their metabolites, as well as in the imaging studies that allow the detection of even small adrenal, extra-adrenal and metastatic lesions, have simplified the diagnosis of PHEOs/PGLs.

Biochemical Diagnosis

Biochemical diagnosis of PHEO requires confirmation by several tests, the most important being biochemical evidence of excessive catecholamine production by the tumour. This is usually achieved by measurements of catecholamines and specific metabolites in the urine or plasma. As many PHEOs secrete catecholamines episodically whereas metanephrines are produced continuously within the tumour cells, fractionated plasma and urine metanephrines (metanephrine and normetanephrine) are the diagnostic tests of choice. Both

plasma and urine measurements have similar sensitivity (96–100%) but the specificity for plasma-free metanephrines is better than urinary fractionated metanephrines (89 versus 69%), so they exclude PHEO in more patients without the tumour and prevent further unnecessary investigation. Another issue is the extent of the elevation in biochemical test results. An elevation of more than fourfold above the reference interval is associated with a nearly 100% probability of the presence of the tumour. Similarly, an increase in urinary normetanephrine >1500 µg/day and in metanephrine >600 µg/day is rare in patients without PHEO [7].

The conditions under which blood or urine samples are collected can be crucial to the reliability and interpretation of test results. Blood samples should be collected with patients supine for at least 15–20 minutes before sampling. To avoid stress associated with venepuncture, samples should be collected through a previously inserted intravenous catheter. The 24 hour urine collection for fractionated metanephrines should include measurements of urine creatinine to verify adequate collection.

Measurement of plasma methoxytyramine can be useful in assessing whether the tumour hypersecretes dopamine, because plasma metanephrine fractions are not direct metabolites of dopamine. Methoxytyramine has been suggested as a good biochemical marker to discriminate patients with *SDHB* and *SDHD* mutations from those with MEN2, NF1 or VHL and recently as a novel biomarker for metastatic PPGLs, but this test is not widely available commercially.

The type of catecholamine and metabolites produced may help localization of tumour and estimate malignancy potential. PHEOs produce both epinephrine and norepinephrine, in contrast to PGLs, which produce almost exclusively norepinephrine. Malignant PHEOs usually produce norepinephrine, as well as high plasma and urine dopamine [7]. It should be noted that several drugs and conditions interfere with the assays used for measurement of catecholamines and metabolites (Table 12.3). Chromogranin A (CgA) is another marker that has been used for the diagnosis of a PHEO and in post-operative follow-up, but it is not specific for PHEOs and it is released from other tissues (e.g. pituitary gland, parathyroid glands, central nervous system [CNS] and pancreatic beta cells).

Table 12.3 Medications and conditions that can interfere with catecholamine measurements.

Class	Compound	Effect
Stimulants	• Caffeine (coffee, tea) • Nicotine (tobacco) • Theophylline	Increase
Sympathomimetics	• Amphetamines • Decongestants (phenylephrine or pseudoephedrine) • Albuterol	Increase
α-Blockers	• Phenoxybenzamine • Doxazosin • Terazosin • Prazosin	Increase
β-Blockers	• Atenolol • Metoprolol • Propranolol • Labetalol	Increase
Calcium channel antagonists	• Nifedipine • Amlodipine • Diltiazem • Verapamil	Increase
Monoamine oxidase inhibitors	• Phenelzine • Tranylcypromine • Selegiline	Increase
Miscellaneous	• Levodopa • Methyldopa • Carbidopa • Cocaine	Increase
Miscellaneous	• Clonidine • Disulfiram • Salicylates • Guanethidine	Decrease

Source: Adopted by Xekouki and Stratakis [10].

Imaging

Once the biochemical diagnosis of catecholamine excess has been established, imaging studies should be performed to localize the tumours. The initial test of choice for children is MRI with or without contrast because it does not involve radiation exposure. MRI is reliable and particularly useful for intracardiac, juxtacardiac or juxtavascular PGLs and for those adjacent to vena cava to detect vascular invasion. T1-weighted images of PHEOs have a signal like liver, kidney and muscle, distinguishing them from cortical adenomas, which contain fat and therefore have an intense bright signal. The hypervascularity of PHEOs makes them appear bright on T2-weighted images with no signal loss on opposed phase imaging but other adrenal malignancies, adrenal adenomas and haemorrhages may also appear bright and further imaging studies may be needed.

PHEO and PGL have specific cellular and intracellular characteristics that favour the use of functional imaging modalities. MIBG has been used for diagnostic imaging in PHEO given its good affinity and uptake by the norepinephrine transporters. Also, it is selectively

accumulated in neurosecretory granules and not metabolized. Scintigraphy using ^{131}I-MIBG or ^{123}I-MIBG is used to locate and confirm PHEOs and rule out metastatic disease. The specificity of MIBG is about 95–100% but this technique offers suboptimal sensitivity (77–90%) with the ^{131}I-labelled agent [7]. Sensitivity of 83–100% is available with ^{123}I-MIBG.

Other advantages with the ^{123}I isotope are superior image quality, lower γ radiation, the ability for single-photon emission CT and the shorter half-life (13 hours versus 8.2 days) so higher doses can be used. Nevertheless, the sensitivity for both isotopes for metastatic disease is lower.

MIBG scanning is more sensitive for benign, unilateral, adrenal and sporadic PHEOs and less sensitive for bilateral, malignant, extra-adrenal and MEN2- and VHL-related PHEOs. It is important to block thyroid uptake of free ^{123}I/^{131}I so saturated solution of potassium iodide (SSKI, 5 drops orally twice a day before the injection and 4 days after) is administered. It is important to note that false negative scans can occur with several medications (Table 12.4). All such agents should be discontinued at least 2 weeks before MIBG scintigraphy.

The positron-emitting compound [18F] may be used in combination with several carrier compounds that are relevant substrates for the norepinephrine transporters. The compound dopamine is a much better substrate for the norepinephrine transporter than norepinephrine, so 18F-fluorodopamine ([18F]-FDA) was developed at the National Institutes of Health (NIH) as an imaging agent for PHEOs/PGLs. Dopa is another agent that has been used to create another imaging compound – 18F-dihydroxyphenylalanine ([18F]-FDOPA) based on the ability of [18F]-FDOPA to be converted to [18F]-FDA, which is subsequently stored in the intracellular vesicles.

The most frequently used PET imaging agent is [18F]-FDG PET. This agent is used based on the excessive glucose uptake of the tumours via GLUT-1 receptors. Although it is not specific for PHEOs/PGLs, studies have shown that it can be useful in patients with these tumours, particularly for those with malignancy potential that are becoming undifferentiated and losing their ability to accumulate more specific agents. Studies have addressed the performance of functional imaging modalities in relation to the underlying mutation. It has been shown that in patients with MEN2-associated PHEO ^{123}I-MIBG scintigraphy, [18F]-FDA PET and [18F]-FDOPA PET can detect equally the presence of the tumour. By contrast, in patients with VHL-associated PHEOs/PGLs, the tumours are better localized with [18F]-FDA PET than with ^{123}I-MIBG scintigraphy.

In a head-to-head comparison between [18F]-FDA, [18F]-FDOPA PET, [18F]-FDG PET/CT and ^{123}I-MIBG for localizing benign and malignant sympathetic PGLs, it was found that the sensitivity for CT and/or MRI (100%) in localizing non-metastatic PGL was 88% for [18F]-FDG PET/CT, 81% for [18F]-FDOPA PET, 78% for [18F]-FDA PET and 78% for ^{123}I-MIBG scintigraphy. For metastatic PGL CT/MRI, [18F]-FDA PET had a higher sensitivity (76%) compared with [18F]-FDG PET/CT (74%), [18F]-FDOPA PET (45%) and ^{123}I-MIBG (57%). Thus, the preferred technique for localization of primary sympathetic PGL and to rule out metastases is [18F]-FDA PET. If this is unavailable, [18F]-FDOPA PET or ^{123}I-MIBG can be used. For patients with known metastatic PGL, the use of [18F]-FDA PET is recommended in patients with an unknown genotype, [18F]-FDG or [18F]-FDA PET in *SDHB* mutation carriers and [18F]-FDOPA PET or [18F]-FDA PET in non-*SDHB* patients. The use of ^{123}I-MIBG scintigraphy in patients with metastatic PGL should be limited to the evaluation of whether the patient qualifies for ^{131}I-MIBG treatment.

Considering the genetic phenotype, [18F]-FDA PET and ^{123}I-MIBG scintigraphy were not able to detect tumours in patients with *SDHB* mutations, whereas [18F]-FDG PET/CT could detect the tumours in all patients with *SDHD* mutations and in only one patient with an *SDHB* mutation. The authors concluded that [18F]-FDOPA PET may be a potential first-line functional imaging agent for the localization of *SDHx*-related head and neck PGLs.

Treatment

The mainstay in the treatment of the hormone-secreting PHEO/PGL is surgical resection by an experienced surgeon. Imaging may follow up non-secreting PGLs in the context of familial syndromes. The frequency and type of biochemical screening and imaging vary, depending on the disease, the mutation (in some cases) and/or the patient and the size and location of the lesion. Genetic counselling should be offered to all patients (and their families) with germline mutation-proven disease and/or clinical diagnosis of an identifiable syndrome.

Medical Treatment and Pre-Operative Management

Pre-operative management should be started 1–2 weeks before surgery to avoid hypertensive crises and cardiac arrhythmias that may arise from acute catecholamine surges during induction of anaesthesia and tumour manipulation. Sympathetic PGLs (rarely parasympathetic) may secrete catecholamines, so appropriate pre-operative preparation should be performed like the classic PHEO. With adequate pharmacological preparation, operative mortality is below 1%. There is no universal algorithm but most authors prefer to combine an α-adrenoreceptor blocking agent with a β-adrenoreceptor blocking agent. The role of alpha blockade is equally

Table 12.4 Drugs potentially affecting metaiodobenzylguanidine (MIBG) uptake.

Drug class	Medication
Central nervous system stimulants (norepinephrine and dopamine reuptake inhibitors)	• Cocaine • Dexmethylphenidate • Methylphenidate • Benzphetamine • Diethylpropion • Phendimetrazine • Phentermine • Sibutramine
Monoamine oxidase inhibitors	• Isocarboxazid • Linezolid • Phenelzine • Tranylcypromine
Central monoamine-depleting agent	• Reserpine
Non-select β-adrenergic blocking agents	• Labetalol
Opioid analgesic	• Tramadol
Sympathomimetics	• Pseudoephedrine • Amphetamine • Dextroamphetamine • Ephedrine • Phenylephrine • Methamphetamine • Phenylpropanolamine • Dopamine • Isoproterenol • Salbutamol • Terbutaline • Phenoterol • Xylometazoline
Tricyclic antidepressants (serotonin/norepinephrine reuptake inhibitors)	• Amitriptyline and derivates • Imipramine and derivates • Amoxapine • Loxapine • Oxepine
Antipsychotics (neuroleptics) (reuptake inhibitors)	• Chlorpromazine • Benperidol • Flupentixol • Fluphenazine • Haloperidol • Levomepromazine • Perphenazine • Pimozide • Prochlorperazine • Promazine • Sulpiride • Thioridazine • Trifluoperazine • Clozapine • Olanzapine • Quetiapine • Risperidone

Source: Adopted by Xekouki and Stratakis [10].

crucial to allow for expansion of blood volume, since these patients are invariably volume depleted. Normotensive patients often become hypertensive during surgery, so these patients should also be given α-blockers pre-operatively.

Surgery and Post-Operative Treatment

Surgery, the treatment of choice, is rarely urgent. It is safer for the patient to have several weeks on alpha blockade than be rushed into surgery within days of diagnosis. Due to the high incidence of bilateral adrenal disease in hereditary PHEOs, partial adrenalectomies (laparoscopic resection of small PHEOs, sparing the adrenal cortex) are advocated to avoid morbidity associated with adrenal replacement therapy. The cortical-sparing approach is particularly attractive in young children who may not comply with lifelong glucocorticoid and mineralocorticoid replacement but such adrenal-sparing techniques can result in tumour recurrence, with a rate of 24%.

Severe shock and cardiovascular collapse can occur in the immediate post-resection period due to withdrawal of hypercatecholaminaemia. Volume replacement with intravenous fluids is mandatory. During the first 24–48 hours, large fluid volumes, often 0.5–1.5 times the patient's blood volume, may be needed. Post-operative hypertension is usually caused by volume overload, pain, autonomic instability or residual tumour.

24 hour urine collections for fractionated catecholamines and metanephrines are obtained at least 2 weeks after surgery, because catecholamine excretion often remains high for up to 10 days after surgery. Thereafter, urine collections are obtained every 3 months for the first year following surgery, then annually or semi-annually for at least 5 years, particularly for tumours >5 cm. Weekly home blood pressure monitoring is recommended for the first year post-operatively and monthly thereafter. A rise in blood pressure or recurrence of symptoms may indicate recurrent or metastatic disease and warrants full workup. Annual MRI or CT of the neck, thorax, abdomen and pelvis should continue indefinitely. The value of CgA as a follow-up marker has been debated. It may be useful in those patients with a baseline elevation at the time of diagnosis.

For children with an identified genetic mutation predisposing to the development of PHEO/PGL, annual screening is advised. The age of initial screening is determined by the specific gene mutation. In MEN2A and MEN2B, it is recommended at 5–10 years; in familial PGL syndromes due to *SDHD*, *SDHC* or *SDHAF2* mutations, it is recommended at 10 years. For syndromes due to *SDHB* mutations, initial screening is recommended at 5 years and in VHL disease also at 5 years.

Management of Malignant Disease

Malignant PHEO is rare in children and the evidence base informing the management of these patients is drawn primarily from the adult literature. Approximately 10% of PHEOs and almost 50% of SDHB-mutated PGLs are malignant. The definition of malignancy in the case of PHEOs/PGLs is established by the presence of metastases to a site where paraganglionic tissue is not normally present (e.g. liver or bone). Distant metastasis can occur through haematogenous or lymphatic routes to LNs, bone, lung and liver. Patients with liver or lung metastases are considered short-term survivors (<2 years). Patients presenting with bone lesions are considered long-term survivors (more than 20 years after diagnosis) [7]. The bones most frequently involved include vertebrae, pelvis, ischium, clavicles, proximal femurs and humeri. SDHB mutant PHEOs and PGLs are particularly prone to bone metastases often in the skull, particularly frontal bones.

Hormonal manifestations of malignant PHEOs/PGLs are like benign lesions with additional symptoms due to tumour mass. Increased concentrations of catecholamines and metanephrines can be found in both benign and malignant tumours but higher concentrations of plasma and urine metanephrines and increased concentrations of dopamine can usually be found in the latter due to larger tumour burden and dedifferentiation [7].

Treatment for metastatic PHEO/PGL is not curative and the main goal is disease stabilization. Resection of the primary mass or metastases should always be considered because it may alleviate symptoms due to tumour mass effect and reduce hormonal activity on the cardiovascular system. It may also facilitate the efficacy of other therapeutic modalities but there is no evidence that it prolongs survival of patients with metastatic disease. Open procedures are preferred. Total adrenalectomy or complete resection of PGLs and resection of regional LNs or distant metastases are recommended. Cryoablation, radiofrequency ablation or arterial embolization can be performed for liver lesions.

^{131}I-MIBG treatment is the first-line systemic treatment for malignant PHEOs in MIBG-positive tumours. The procedure is usually well tolerated with minimal toxicity, which includes nausea, occasional sialadenitis, mild bone marrow suppression (most frequently thrombocytopaenia), mildly elevated liver enzymes and renal toxicity. There is some risk for long-term toxicity that needs to be taken into consideration, which includes infertility (sperm/oocyte cryopreservation is suggested before treatment) and an increased lifetime risk of second malignancies, particularly myelodysplasia and leukaemia. Treatment with ^{131}I-MIBG should not be considered curative but it can serve as an adjuvant

therapy following tumour debulking and facilitate disease stabilization.

In rapidly progressive disease, chemotherapy rather than ^{131}I-MIBG treatment is recommended. The most effective regimen appears to be the combination of cyclophosphamide, vincristine and dacarbazine (CVD), which, given in 12 patients every 21 days, caused complete or partial remission in 57% of patients. In another study 18 patients with metastatic disease were followed for 22 years: complete remission was found in 11% of patients and partial remission in 44%. The median overall survival of the responders was 3.8 years compared with 1.8 years of non-responders. CVD, like surgery, can induce hypertensive crisis within the first few hours of administration, particularly in patients with large tumour burden, and administration of a-methyl-p-tyrosine before chemotherapy to inhibit catecholamine synthesis is recommended. External beam radiation is only used as palliative therapy for chronic pain and symptoms of local compression arising from local metastases.

With the identification of the genetic events that lead to the development of endocrine tumours, novel agents known as targeted therapies have been developed. These cytostatic therapies target specific cell signalling molecules that, when mutated, lead to enhanced cell growth or failure to undergo normal cell death. Examples include mutations in genes encoding tyrosine kinase receptor(s) such as RET, PDGFR, KIT and epidermal growth factor. Tyrosine kinase inhibitors (TKIs) are small organic molecules that interfere with the interaction between the kinase domain and ATP or inhibit phosphorylation of the kinase and downstream substrates such as rat sarcoma (RAS) and serine/threonine protein kinase B-raf (BRAF). Sunitinib and sorafenib are potent oral TKIs that inhibit phosphorylation of PDGFRA, KIT, VEGF receptors 2 and 3 and RET. Data are lacking on the role of these compounds in pediatric patients and, given the associated toxicity, their use should be restricted to clinical protocols.

Adrenocortical Cancers

Adrenocortical Cancers (ACC) are rare in children. The first case was reported in 1865 and, until 1937, most children undergoing adrenalectomy for a functioning ACC would die post-operatively of adrenocortical insufficiency. When cortisone became available, outcome improved dramatically. Childhood ACC have distinct clinical and biological features that contrast with those observed in other pediatric carcinomas. The incidence of most childhood carcinomas increases with age, whereas 65% of ACC occur in children younger than 5 years of age. In fact, this age distribution resembles that of tumours of embryonic origin. Moreover, most children with ACC appear healthy and usually have normal development. Finally, smaller-sized ACC can cause exuberant endocrine syndromes that often mask or delay the diagnosis.

Epidemiology

ACC is rare. In a report of the SEER programme, 28 cases of adrenocortical carcinomas in patients younger than 20 years were reported between 1973 and 1987. From these data, it is estimated that there are 19 new cases of adrenocortical carcinomas per year in the USA in this age group but the true incidence of adrenocortical carcinoma may be higher. The estimated figures do not account for those cases of adrenocortical carcinomas that are misdiagnosed as adenomas and therefore not reported. If it is assumed that one-third of all ACC are adenomas, an estimated number of 25–30 cases of ACC occur annually in patients under the age of 20 years in the USA.

Particularly remarkable is the incidence of ACC in southern Brazil. The annual incidence of ACC within this region can be estimated to range from 3.4 to 4.2 per million children below the age of 15 years. By comparison, the annual worldwide incidence of childhood ACC ranges from only 0.3 to 0.38 per million children below the age of 15 years. Explanations for this apparent excess of ACC cases in southern Brazil are lacking. The region is located below the Tropic of Capricorn and has no known endemic transmissible diseases. The population is mainly of European extraction – Italy, Poland and Germany – locales in which the incidence of ACC in children has not been unduly increased. Moreover, as compared with other regions of Brazil, the southern states have had the least native Indian influence during colonization. A genetic predisposition towards cancer, which appears to play a role in many childhood tumours, is not a common feature among southern Brazilian families of children with ACC but multiple cases of childhood ACC have been noted in some families, suggesting a possible genetic basis for this disease [21].

Pathogenesis

Predisposing constitutional genetic factors have been found in ~50% of children with ACC. Two genetic syndromes are clearly associated with ACC: Li–Fraumeni syndrome, which is associated with alterations of the tumour suppressor gene *TP53* on chromosome 17p [22, 23], and Beckwith–Wiedemann syndrome, associated with alteration of the 11p15 region.

In families with the Li–Fraumeni syndrome, the frequency of ACC is 100 times that of the general population. The Li–Fraumeni syndrome is a rare autosomal dominant condition with incomplete penetrance in which affected members develop many different types of tumours. In addition to childhood sarcoma and premenopausal breast cancer, members of these families have an increased risk for the development of other malignancies, including leukaemia, brain tumours, osteosarcomas and adrenocortical carcinomas.

Beckwith–Wiedemann syndrome occurs with a prevalence of 1 in 1300. The mode of inheritance is complex. Possible patterns include autosomal dominant inheritance with variable expressivity, genomic imprinting resulting from a defective or absent copy of the maternally derived gene and contiguous gene duplication at 11p15. The cardinal features are exomphalos, midline abdominal wall defects, macroglossia, neonatal hyperinsulinaemia and macrosomia. Ear pits and ear creases are also commonly observed. Apart from ACC, patients are at increased risk for other malignant and benign tumours including Wilms tumour, hepatoblastoma, neuroblastoma and rhabdomyosarcoma.

After the early discovery of the role of TP53 in ACC development, no more breakthroughs and molecularly designed therapies in adrenocortical cancer were developed. As early as 2003, the suggestion was made that adrenocortical cancer is not different from other solid tumours in terms of its formation and progression but lack of access to samples in which the hyperplasia–adenoma–carcinoma sequence was evident impeded further investigation of this hypothesis. The hypothesis of an adenoma–carcinoma sequence in adrenocortical cancer was tested in 2013 by Custodio et al. [24] who preclinically detected carriers of the most frequent low-penetrance *TP53* mutant allele, the p.Arg337His. In this first ever study of newborn genetic screening for a cancer-predisposing genetic defect, 171,649 newborns in the state of Parana, Brazil, where this mutation is particularly frequent, were screened between 2005 and 2010. A total of 461 infants carried the p.Arg337His allele (0.27%) and 11 of them developed a tumour during the study compared with only two from the remaining 171,188 children.

One patient had a benign adrenocortical adenoma at surgery but most had small tumours with stage I cancer. These findings implicate an adenoma–carcinoma sequence in adrenocortical cancer and this process was further supported by a single nucleotide polymorphism (SNP) array study published in 2013 when Ronchi et al. [25] showed that malignant tumours have more genomic aberrations than benign ones. The SNP array findings also confirmed the involvement of IGF2 in advanced adrenocortical cancer and showed the heterogeneity of the samples studied. Gene network analysis by Ronchi et al. [25] identified Wnt signalling as the most relevant network in adrenocortical cancer, indicated by the involvement of WNT5A signalling and many related genes (*APC2*, *AXIN1*, *NKD2* and others) in adrenocortical cancer. Evidence from this study and several others suggest that dysregulation of the β-catenin pathway and Wnt signalling could lead to tumour formation or progression in adrenocortical cancer from, possibly, resident stem cells. These studies suggest that Wnt signalling inhibitors could be used in the treatment of adrenocortical cancer and, for any drug therapy to succeed, it must be applied after the molecular signature of the adrenocortical cancer of the individual patient is characterized at the genetic and epigenetic level [26].

Clinical Presentation

The presenting manifestations of ACC depend largely on the tumour's secretion of adrenocortical hormones (Table 12.5) [22]. Hormone-secreting tumours and the associated classic endocrine syndromes (virilizing, feminizing, Cushing and Conn syndromes) represent the most common presentation in this age group. ACC usually secrete several hormones and thus present signs and symptoms of multiple syndromes (mixed forms). Nonfunctional tumours, the most common type in adults, comprise ~10% of the pediatric/adolescent cases.

The virilizing syndrome (deepening of the voice, acne, hirsutism, increase of muscle mass and secretion and proliferation of the sebaceous glands with characteristic

Table 12.5 Signs and symptoms of adrenocortical tumours in 58 children.

Feature	Number	%
Pubic hair	53	91
Hypertrophy: clitoris/penis	36/13	62/22
Acne	42	72
Deep voice	32	55
Hypertension	32	55
Facial hair	29	50
Facial hyperaemia	28	48
Palpable tumour	28	48
Weight gain	22	38
Hirsutism	21	36
Moon face	19	33
Accelerated growth velocity	17	29
Centripetal fat distribution	14	24
Buffalo hump of the neck	11	19
Seizures	7	12

adult smell) is the most common presentation in this age group, corresponding to 80% of all patients. Gender-specific changes may be noted: in females, clitoral enlargement, facial and pubic hair with male escutcheon, amenorrhoea and rarely temporal balding are the most common with penile enlargement and precocious isosexual pseudo-puberty in males.

Cushing syndrome (moon facies, weight gain, centripetal distribution of fat [abdomen and upper dorsal region], plethora, hypertension and striae) occurs in approximately one-third of the patients but isolated hyperadrenocorticalism in only 8%. Most had signs and symptoms mixed with those from virilizing syndrome.

Conn syndrome or primary aldosteronism is commonly seen in bilateral cortical hyperplasia. Aldosterone-producing adenomas are very rare in children (<2% of all ACC). Headache, weakness of proximal muscle groups, polyuria, tachycardia with or without palpitation, hypocalcaemia and hypertension are the most common presenting clinical features. Like Cushing syndrome, the signs and symptoms of Conn syndrome can be obscured by other manifestations. Feminization is a rare form of presentation accounting for 2.2% of the cases. The most frequent sign is gynaecomastia.

Children and adolescents with a functioning ACC are subject to growth disturbances. Pure androgen and oestrogen excess most often results in increased growth rate and premature epiphyseal closure. It is not clear if this abnormal exposure to adrenal hormones will compromise final adult height. Some investigators suggest that it will result in shorter than expected final adult height but others have found a tendency to tall rather than short stature in nine children treated by surgical excision of ACC. In many instances, the increased somatic growth of these children, their generally healthy appearance and the lack of a palpable abdominal mass diverted pediatricians from the possibility of a malignancy. The resulting delay in diagnosis can be appreciated from the long median interval (10 months; range, 3 days to 61 months) between the first clinical manifestations of ACC and its diagnosis [22]. To avoid delaying diagnosis of ACC, any child <4 years with pubarche should be considered to have an ACC until proven otherwise. In addition, the presence of acne in an infant can be considered pathognomonic of an adrenocortical lesion. Finally, because Cushing syndrome is very rare in children, it should be considered highly indicative of ACC in children younger than 10 years of age.

Diagnosis

ACC arise from one of three adrenal cortex layers: glomerulosa, fasciculata and reticularis. It has classically been considered an epithelial tumour and therefore classified as carcinoma or adenoma. The diagnosis of an ACC is made based on the gross and histologic examination of the surgical specimen.

The pathologic classification of pediatric ACC is troublesome even for the most experienced pathologists. Bugg et al. [23] applied a modified criterion of Weiss and colleagues to analyse a large series of pediatric ACC. In this study, adrenal tumours were divided into adrenocortical adenomas and high-grade or low-grade carcinomas. This classification was based on the mitotic index, confluent necrosis, atypical mitoses and nuclear grade. High-grade carcinoma and tumour weight were the most reliable predictors of outcome.

Measurements of urinary 17-ketosteroids (17-KS) frequently provide the pivotal clue to a diagnosis of ACC. The 17-KS levels are elevated in most patients. Plasma dehydroepiandrosterone sulphate (DHEA-S) concentrations are elevated in ~90% of the cases, suggesting that increase in plasma DHEA-S levels is the second most reliable tumour marker. Abnormal urinary DHEA concentrations are less sensitive, occurring in only 2/3 of patients. Urinary 17-hydroxycorticosteroid (17-OH) levels are elevated in cases with clinical signs of excess glucocorticoids. A dexamethasone suppression test was rarely necessary. The presence of elevated concentrations of glucocorticoid and androgen is a strong indication of an adrenal tumour.

Routine laboratory evaluation for patients suspected of having ACC includes measurement of urinary 17-KS, 17-OH progesterone and free cortisol, as well as plasma cortisol, DHEA-S, testosterone, androstenedione, 17-hydroxyprogesterone, aldosterone, renin activity, DOC and other 17-deoxysteroid precursors. This comprehensive panel of tests not only contributes to the diagnosis but also provides useful markers for surveillance for tumour recurrence after radical treatment.

Several imaging modalities have been used to establish the diagnosis of ACC including ultrasound, CT and MRI. Ultrasound is the first choice for the definition of tumour location, dimensions and characteristics and for evaluating tumour extension into the inferior vena cava and right atrium. All patients suspected to have an adrenal tumour should be examined by CT or MRI. In most institutions, MRI has steadily increased because it has several advantages over CT, including lack of ionizing radiation, capability of imaging multiple planes and improved tissue contrast differentiation. Moreover, MRI may discriminate between benign and malignant lesions.

Treatment

Surgery

Surgery is the most important procedure in the treatment of ACC. Because of tumour friability, rupture of the

capsule and tumour spillage are frequent (occurring in ~20% of cases during the initial procedure and in 43% after local recurrence). Infiltration of the vena cava can be expected to make radical surgery difficult in some cases, although successful complete resection of the tumour thrombus has been reported in patients undergoing cardiopulmonary bypass. Surgery requires careful and precise perioperative planning. All patients with a functioning tumour are assumed to have suppression of the contralateral adrenal gland, so steroid replacement therapy is mandatory. Special attention to electrolyte balance, blood pressure, wound care and infectious complications is imperative.

Chemotherapy

The role of chemotherapy in the management of childhood ACC has not been established. Mitotane (1,1-dichloro-2-[0-chlorophenyl]-2-[p-chlorophenyl]ethane, or o,p'-DDD), an insecticide derivative that produces adrenocortical necrosis, has been used extensively in adults with ACC but its efficacy in children is not known. In low doses mitotane inhibits glucocorticoid biosynthesis and, in high doses, it induces cell death in the zona fasciculata. The zona glomerulosa is also affected but less severely so. Mitotane accelerates the metabolic clearance of glucocorticoids, thyroid and parathyroid hormones so glucocorticoid and mineralocorticoid replacement therapy is required in all patients and many also require thyroid hormone supplementation.

The role of mitotane in the management of ACC is uncertain. Up to 30% of patients with advanced disease have an objective response but this is usually transient and the effect on survival is unknown. Complete responses have been reported in children with advanced or metastatic disease but appear to be unusual. The most important toxicities have been gastrointestinal and neurological, including nausea, vomiting, diarrhoea and abdominal pain in a high proportion of patients. Less frequent reactions included somnolence, lethargy, ataxic gait, depression and vertigo. Of interest, all prepubertal patients developed gynaecomastia or thelarche.

Another shortcoming of mitotane treatment is that it significantly alters steroid hormone metabolism, so steroid measurements in blood and urine cannot be used as a marker of tumour relapse. Therefore, due to lack of efficacy data and substantial toxicities, mitotane should be considered an experimental agent in the treatment of children with ACC.

Other antineoplastic drugs, including the combination of cisplatin with etoposide, 5-fluorouracil with leucovorin and ifosfamide and carboplatin with etoposide, have been used in too few patients to permit meaningful conclusions. Adjuvant radiation therapy has not yet been evaluated in childhood ACC.

Table 12.6 Staging of adrenocortical tumours.

Stage I	Total excision of tumour, tumour volume <200 cm^3 *and* absence of metastases and normal hormone concentrations after surgery
Stage II	Microscopic residual tumour, tumour volume >200 cm^3 *and* persistently elevated adrenocortical hormone concentrations after surgery
Stage III	Gross residual or inoperable tumour
Stage IV	Distant metastasis

Source: Revised from the previous edition of the book.

Staging and Prognosis

The staging system for ACC is given in Table 12.6. Prognosis is closely related to the stage of the tumour at diagnosis, with 91% of the children presenting with stage I disease and 53% of those with stage II disease being recurrence-free 5 years from diagnosis. Very few children present with stage III or IV disease but their prognosis is extremely poor.

Data from the International Pediatric Adrenocortical Tumors Registry reported that 44.1% of children had stage I disease at diagnosis and 31.5% had stage II disease and the mean duration of symptoms prior to diagnosis was 5 months [27]. Prognosis is more favourable in children who present below the age of 3 years, those with tumour weighing <80 g, and isolated virilization at presentation.

Tumours of the Ovary

Ovarian neoplasms (benign and malignant) account for ~1% of all tumours in children and adolescents. Most are physiologic or benign, fewer than 5% of ovarian malignancies occurring in this age group. Ovarian enlargement, whether cystic or solid, must be evaluated to exclude malignancy because ~10–20% of ovarian masses occurring during childhood and adolescence are malignant. Germ cell tumours (GCTs) comprise one-half to two-thirds of ovarian neoplasms in girls up to 18 years of age, and ovarian sex cord stromal tumours (SCSTs) another 15%, while epithelial tumours are rare especially in the prepubertal age group and will not be reviewed here.

Ovarian Germ Cell Tumours (GCTs)

These tumours account for about 30% of GCTs and 70% of all neoplastic ovarian masses, being the most common ovarian neoplasms in children and teenagers. The peak of incidence is in early adolescence [28].

Pathology

All the histological subtypes of GCTs may be represented in the ovary. Benign and immature teratomas constitute about 80% of ovarian GCTs and are bilateral in 5% of cases, whereas the incidence of malignant forms is reported in about 20% and increases during adolescence. The most common malignant entity in the young population is the yolk sac tumour (YST); dysgerminoma is the most frequent ovarian malignant GCT during adolescence and may be bilateral in 10% of cases. Gonadoblastoma is a rare GCT observed in girls with 46,XY disorders of sex development (DSD) with dysgenetic gonads. The tumour exhibits benign behaviour but may be associated with malignant histotypes. For this reason, bilateral gonadectomy is recommended in cases with dysgenetic gonads to prevent the development of malignant changes [28].

Clinical Presentation

The clinical presentation can be similar in both malignant and benign tumours; abdominal pain (70–80%) and lower abdominal mass are the most common symptoms. Constipation, amenorrhoea and vaginal bleeding are less frequent but tumours are often asymptomatic until they reach a very large size. Tumours with trophoblastic cells may produce βHCG that causes precocious breast enlargement and pubic hair. The pattern of regional spread of tumours with malignant components often includes ascites and peritoneal metastases. About 10% of cases present as acute abdomen due to torsion, infarction or spontaneous rupture of the mass.

Diagnostic Evaluation

This includes an abdominal ultrasound that typically shows a solid/cystic lesion. Abdominal CT/MRI scans are necessary to define the size, the structure of the mass and the involvement of neighbouring structures. Benign and malignant entities may have similar imaging features with solid and cystic components but a cystic component is more frequent in benign forms.

Evaluation of serum markers is essential for both the diagnosis and surveillance of these tumours. Alpha-fetoprotein (αFP) is elevated in over 90% of children with malignant GCTs and βHCG in 30% of cases. Other markers such as lactate dehydrogenase (LDH) and those characteristic for epithelial cell tumours (carcinoembryonic antigen [CEA], cancer antigen 125 [CA-125]) may be slightly elevated in patients with mixed tumours [12].

Surgery for Ovarian GCTs

The only effective therapy for benign and immature GCTs is complete removal. If histological completeness has not been achieved at primary surgery, a reoperation is recommended. The procedure often requires ovariectomy due to the size of the mass or uncertainty of diagnosis. In selected cases with small tumours, enucleation of the lesion may be feasible and many surgeons prefer ovary-sparing operations, especially for unilateral lesions. Every effort should be made to preserve hormonal and reproductive functions in patients with bilateral benign GCTs. If the diagnostic suspicion for malignancy is low and the surgeon is expert in minimally invasive surgery, a laparoscopic approach may be considered in cases with small or cystic masses [29].

Surgery is critical for the management of girls with malignant GCTs. The surgical approach can be performed through a Pfannenstiel or transverse infraumbilical incision or through a midline approach, depending on the size of the lesion and the likelihood of malignancy. Whatever the approach, the surgeon should be prepared to perform an oncologic operation that should include well-defined intraoperative staging [30]:

1) Collection of peritoneal fluid for cytology (or washing if no fluid is present).
2) Inspection and palpation of the contralateral ovary with excision of suspected lesions.
3) Intact removal of the ovary without rapture of the tumour capsule.
4) Palpation of the omentum, the peritoneal surface and liver with removal of any abnormal areas. Peritoneal implants (gliomatosis peritonei) may be associated with mature or immature teratomas.
5) Palpation of the iliac and aorto-caval nodes with biopsy of any abnormal nodes.

Staging of Malignant GCTs

There are two main systems adopted for pediatric ovarian malignant tumours: the staging system suggested by the Children's Oncology Group (COG) is summarized in Table 12.7 and that from the International Federation of Gynaecology and Obstetrics (FIGO) standardized for adult and pediatric patients in Table 12.8. Both are based on the results of imaging and surgical pathology.

Ovarian Sex Cord Stromal Tumours (SCSTs)

These tumours are distinct from GCTs and comprise ~7% of ovarian tumours overall and ~15% of ovarian tumours in children. Early in embryonic life, the sex cords arise from the primitive genital ridge or coelomic epithelium. In females, the sex cords develop into the cortical cords and later into ovarian follicles. SCSTs arise from the sex cords or from ovarian stroma/mesenchyme of the developing gonad and include both juvenile-type and adult-type granulosa cell tumours, Sertoli–Leydig cell tumours, Sertoli cell and Leydig cell tumours and theca and granulosa-theca tumours,

Table 12.7 Staging of pediatric ovarian germ cell tumours according to the American Pediatric Oncology Group and Children's Cancer Group.

Stage I	Limited to ovary (ovaries) – peritoneal washings negative for malignant cells; no clinical, radiographic or histological evidence of disease beyond the ovary; tumour markers normal after appropriate half-life decline (αFP, 5 days; βhCG, 16 days)
Stage II	Microscopic residual disease or disease in lymph nodes <2 cm; peritoneal washings negative; tumour markers positive or negative
Stage III	Gross residual disease or biopsy only; lymph nodes >2 cm; contiguous spread to other organs; peritoneal washings positive
Stage IV	Distant metastases

Source: Adopted from the previous edition of the book.

Table 12.8 FIGO staging system for primary ovarian carcinoma.

Stage I	Growth limited to the ovaries
IA	Growth limited to one ovary, no tumour on the external surface; capsule intact
IB	Growth limited to both ovaries, no tumour on the external surface; capsule intact
IC	IA or IB but with ascites or peritoneal washings containing malignant cells; tumour on the surface or capsule ruptured
Stage II	Growth involving one or both ovaries with pelvic extension
IIA	Extension to the uterus or tubes
IIB	Extension to other pelvic structures
IIC	Tumour either IIA or IIB but with ascites or peritoneal washings containing malignant cells; tumour on the surface or capsule ruptured
Stage III	Tumour involving one or both ovaries with peritoneal implants outside the pelvis or positive retroperitoneal or inguinal lymph nodes; superficial liver metastasis equals stage III; tumour is limited to the true pelvis but with histologically proven malignant extension to small bowel or omentum
IIIA	Tumour grossly limited to the true pelvis with negative nodes but histologically proven microscopic seeding of the peritoneal surfaces
IIIB	Tumour of one or both ovaries with histologically confirmed implants of abdominal peritoneal surfaces, none exceeding 2 cm in diameter
IIIC	Abdominal implants are >2 cm in diameter or positive retroperitoneal or inguinal lymph nodes
Stage IV	Growth involving one or both ovaries with distant metastases; if pleural effusion present, there must be positive cytology to allot a case to stage IV; parenchymal liver metastases equals stage IV

Source: Adopted from the previous edition of the book.

sclerosing stromal tumours, SCSTs with annular tubules and gynandroblastomas with simultaneous Sertoli and granulosa cell differentiation (Table 12.9). In children, juvenile granulosa cell tumours are most common followed by Sertoli–Leydig cell tumours. In adults, adult granulosa cell tumours are most common. The recently described microcystic stromal tumour of the ovary is also probably within this category, although to date the youngest patient reported with this diagnosis was 26 years of age [32].

Pathology

Histopathologic diagnosis of ovarian SCST may be challenging and consultation is recommended. Most (~95%) of ovarian SCST stain focally positive for inhibin, although some granulosa cell tumours and the recently described microcystic stromal tumours may be inhibin negative [30]. The finding of inhibin positivity may help differentiate SCSTs from small cell carcinomas of the hypercalcaemic type and other epithelial neoplasms. As the mitotic activity may have a prognostic impact, the mitotic count should be evaluated. This should be supplemented by the immunohistochemical evaluation of proliferation markers such as Ki-67, which still require prospective evaluation.

SCSTs generally have co-expression of cytokeratin and vimentin. They are usually epithelial membrane antigen negative and calretinin positive. In addition, nearly all sex cord tumours are CD56 positive and the staining is usually diffuse and predominantly membranous [33]. Charcot–Bottcher filaments are a distinguishing feature of Sertoli cells and may be present in Sertoli cell tumours or Sertoli–Leydig cell tumours. Histopathologic assessment of Sertoli–Leydig cell tumours should include description of the grade of differentiation, the presence of a retiform subtype and documentation of heterologous elements, all of which are considered prognostically unfavourable.

Clinical Presentation

SCSTs may present in young girls with signs and symptoms like those of GCTs, such as abdominal pain or distention, gastrointestinal symptoms or abdominal mass. These patients often have clinical signs of sex hormone production and may present with isosexual precocity including breast swelling and vaginal bleeding, primary or secondary amenorrhoea and/or virilization.

Juvenile granulosa cell tumours are seen in young children and may present with precocious puberty. Most juvenile granulosa cell tumours are localized (i.e. stage I) at diagnosis and associated with a favourable prognosis. Juvenile granulosa cell tumours rarely recur and, if this occurs, it is generally within the first 2–3 years after diagnosis [34]. Adult granulosa cell tumours are very

Table 12.9 Comparison of ovarian SCST subtypes [31].

	Juvenile granulosa cell tumours	'Adult-type' granulosa cell tumours	Sertoli–Leydig cell tumours	SCST with annular tubules
Typical age	Prepubertal	Post-pubertal	Any age	Teens or later
Clinical manifestations	Precocity	Precocity or menstrual irregularities	Virilization	Precocity or virilization
Associations	Enchondromatosis PJS, DICER1	None	MNG, DICER1	PJS
Tumour markers	May secrete oestrogens, inhibin, MIS	May secrete oestrogens, inhibin, MIS	Testosterone androstenedione	Progesterone, oestradiol, testosterone androstenedione

Source: Reproduced with permission of Wolters Kluwer Health, Inc.
MIS, Müllerian inhibitory substance; MNG, multinodular goitre; PJS, Peutz–Jeghers syndrome; SCST, sex cord stromal tumours.

rare in children. Granulosa cell tumours of both types may secrete inhibin.

Sertoli–Leydig cell tumours generally occur in adolescents and young women. In rare instances, pure Sertoli cell tumours occur and may be predominantly oestrogenic and may produce renin leading to hypertension. Pure Leydig cell tumours have been rarely reported and, when they occur, are predominantly androgen secreting.

Recurrence of stage I ovarian SCST in children is uncommon. When it occurs, recurrence is within a few years of diagnosis (median 2.8 years), most often within the abdominopelvic area or in regional LNs. Haematogenous spread to the chest, liver or bones has been described rarely, most commonly in those with positive LNs at the time of initial surgery and in those with adult granulosa cell tumour histology. In children with localized disease at presentation, later development of metastatic disease outside the abdomen/pelvis is very unlikely [35].

Like any patient with a suspected ovarian tumour, serum tumour markers, αFP, hCG, CEA, CA-125 and LDH should be obtained. For patients with SCSTs especially, serum calcium may help to distinguish small cell carcinoma of the hypercalcaemic type, a non-stromal ovarian carcinoma that may present in childhood or adolescence. Ovarian granulosa cell tumours generally produce inhibin A and inhibin B, both of which may be useful in diagnosis and in follow-up, but the role of these measurements has yet to be fully evaluated. Measurement of inhibin B is more specific and may be more readily available than inhibin A. The wide range of normal in prepubertal children may lead to some uncertainty but, when elevated, inhibin A and B may be useful markers for follow-up. Ovarian SCSTs may also produce Müllerian inhibitory substance. Measurement of testosterone and androstenedione should be performed when virilizing signs or symptoms are present and should be considered even in the absence of obvious clinical symptomatology [36].

Genetic Syndromes

Ovarian SCSTs are associated with multiple enchondromatosis (Ollier disease) and with Peutz–Jeghers syndrome (PJS). Patients with enchondromatosis may develop granulosa cell tumours. Ovarian tumours in PJS are generally of specific histology: SCST with annular tubules. Tumours in patients with PJS tend to present at a younger age and may be bilateral. Patients with PJS may also develop granulosa cell tumours. PJS is associated with germline mutations in the *STK11/LKB1* tumour suppressor gene located on chromosome 19p13.3. LOH at this location was seen in 41% of presumed sporadic SCST in 1 series but no mutations or promoter methylations of STK11 were seen in these tumours [37].

Ovarian SCSTs, particularly Sertoli–Leydig cell tumours but also juvenile granulosa cell tumours and gynandroblastomas, are also seen in families with a history of pleuropulmonary blastoma. *DICER1* mutations are common in children and families with a history of pleuropulmonary blastoma and have been observed in children and adolescents with ovarian Sertoli–Leydig cell tumour, juvenile granulosa cell tumours and/or gynandroblastoma and a personal or family history of pleuropulmonary blastoma or other conditions seen in this familial syndrome, such as renal tumours, lung cysts, additional ovarian tumours, sarcomas or nodular thyroid disease [38]. Ovarian Sertoli–Leydig tumours are also known to be associated with thyroid disease and this association has been reported in conjunction with *DICER1* mutations. All children and adolescents with ovarian tumours should be carefully screened for a personal or family history of dysplastic or neoplastic conditions.

Surgery and Staging for SCSTs

The principles of local therapy and staging are the same as for GCTs of the ovary.

Chemotherapy

Most authors do not recommend further treatment after the complete removal of either a germ cell or a SCST of the ovary in the absence of intraperitoneal dissemination and decrease of tumour markers (stage I). For patients with incomplete removal or recurrent or metastatic disease, systemic chemotherapy is recommended. The treatment utilized by COG suggests PEB (cisplatin, etoposide, bleomycin) ×3 cycles for stage II–III patients (intermediate-risk tumours) and PEB ×4 cycles for those with stage IV disease. The outcome is excellent for patients with locoregional disease (stage I–III) as well as for patients with metastatic disease who may have a cure rate of 50–90% [39].

Tumours of the Testes

Testicular tumours account for 1% of all pediatric solid tumours and occur with an incidence of 0.5–2.0 per 100,000 boys. Two peaks in presentation are observed, the first during the first 2 years of life and the second after the age of 10 years.

Testicular tumours affecting the prepubertal boy differ markedly from those seen in older boys and adult men, both in terms of tumour type and tumour behaviour. GCTs account for 60–70% of tumours in children, while 95% of testicular tumours are of germ cell origin in adults. Seminomas and embryonal carcinomas are not observed in prepubertal boys, while teratomas, which are uniformly benign in prepubertal boys, are often malignant in adults. SCSTs account for 7–8% of childhood tumours. Prepubertal testicular tumours are classified according to the cell line of origin and a modified version of the WHO classification of prepubertal testicular tumours is given in Table 12.10.

Testicular Germ Cell Tumours (GCTs)

GCTs of the testis represent about 10% of all pediatric GCTs but about 30% of malignant GCTs. Bilateral forms are extremely rare. Testicular GCTs have two age peaks: children under 3 years may experience both mature teratoma and malignant GCTs represented almost exclusively by Yolk sac tumours (YST), while adolescents may have also seminomas or other mixed tumours often with delayed diagnosis and more advanced disease [40].

The occurrence of GCTs is increased in patients with undescended testes and the risk is higher with intra-abdominal testes. The most common histological subtype is seminoma, which occurs in adolescents or young adults. The effect of orchidopexy on testicular cancer is not known.

Clinical Presentation

The main clinical feature is a painless scrotal mass but the tumour may develop pain, inflammatory characteristics and hydrocele. Differential diagnosis includes testicular torsion, epididymo-orchitis or post-traumatic haematoma. Paratesticular rhabdomyosarcoma should be considered in the diagnostic workup.

In a patient with a testicular mass, serum marker measurements represent the first diagnostic step to verify a possible malignant GCT. Scrotal ultrasound is the preferred imaging modality to evaluate the lesion. Sometimes it may be difficult to differentiate a testicular mass from a lesion of the paratesticular tissues. Teratomas are usually cystic or partially solid and multicystic, whereas YST is solid. If the diagnosis of a testicular neoplasm is highly likely based on physical examination and initial workup, no other imaging study or biopsy is recommended. The next step is the surgical removal of the tumour as soon as possible. In patients with a suspected malignant GCT at diagnostic workup, thoraco-abdominal CT is recommended to evaluate possible metastatic spread, especially to the lungs and the retroperitoneal LNs.

Surgery

Surgery is the cornerstone of the management of testicular GCTs. Ongoing protocols recommend an inguinal approach with vascular control before mobilization of

Table 12.10 Classification of prepubertal testicular tumours.

Germ cell tumours	Yolk sac, teratomas, seminoma and mixed germ cell
Gonadal stromal tumours	Leydig cell, Sertoli cell, Granulosa cell and mixed gonadal stromal cell
Gonadoblastoma	
Tumours of supportive tissue	Fibroma, fibrosarcoma, leiomyoma, haemangioma
Leukaemia and lymphomas	
Tumour-like lesions	Epidermoid cysts, testicular adrenal rest tumours (TART)
Metastatic tumours	
Tumours of the adnexa	Rhabdomyosarcoma, fibroma, fibrosarcoma, leiomyoma, leiomyosarcoma, haemangioma, lipoma

Source: Adopted from the previous edition of the book.

the testis. If a malignant GCT is proven by frozen section examination, en bloc resection of testis and spermatic structures with ligation of the cord at the internal inguinal ring is required. Complete removal of the tumour is feasible in most cases and the post-operative decrease of αFP will confirm the absence of metastatic spread. Patients with scrotal skin involvement and those operated or biopsied through a scrotal approach should undergo hemiscrotectomy to ensure local control. Primary orchiofunicolectomy is recommended also when distant metastases have been diagnosed. Primary retroperitoneal LN dissection is not recommended in prepubertal boys, since malignant GCTs are highly chemosensitive [41]. Limited biopsy may be necessary to define the staging when the involvement of retroperitoneal LN is uncertain on imaging. Retroperitoneal LN dissection may be required when enlarged nodes remain after chemotherapy [30].

If a benign GCT is the primary diagnosis, based on tumour markers and imaging, testis-sparing surgery through an inguinal approach should be considered. The feasibility of a conservative excision of the tumour mass depends upon its size and site in the gonad. In case of gross residual disease with positive margins in pathology examination of the specimen, a re-excision with inguinal orchiectomy is strongly recommended.

Staging and Systemic Chemotherapy

According to the COG staging system for childhood testicular GCTs, staging can be done after initial surgery (Table 12.11). Excellent survival is reported with surgery alone in stage I patients. They are managed by clinical and αFP monitoring. About 15% develop clinical evidence of relapse, mainly in the retroperitoneal nodes, and can be successfully treated with further multidisciplinary treatment. Chemotherapy is recommended in patients with incomplete initial surgery or with distant metastases at diagnosis. The suggested combinations are the same as those recommended for ovarian GCTs, mainly based on cisplatin-containing regimens. COG suggests PEB × 3 cycles for stage II–IV patients [30].

Table 12.11 Staging of pediatric testicular germ cell tumours according to the American Pediatric Oncology Group and Children's Cancer Group.

Stage I	Limited to testis, completely resected by high inguinal orchidectomy; no clinical, radiographic or histological evidence of disease beyond the testis; tumour markers normal after appropriate half-life decline (αFP, 5 days; βhCG, 16)
Stage II	Trans-scrotal orchidectomy; microscopic disease in scrotum or high in spermatic chord (<5 m from proximal end); retroperitoneal lymph node involvement (<2 m) and/or increased tumour markers after appropriate decline)
Stage III	Tumour-positive retroperitoneal lymph nodes >2 m in diameter: no visceral or extra-abdominal involvement
Stage IV	Distant metastasis

Source: Adopted from the previous edition of the book.

Testicular Stromal Cell Tumours (SCTs)

Prepubertal testicular tumours account for ~1% of pediatric solid tumours. Of these, 11% are SCSTs. Review of data from the Prepubertal Testicular Tumor Registry, a previously open registry study through the American Academy of Pediatrics Urology Section, suggests that most testicular tumours in children behave in a benign manner with the notable exceptions of YST and undifferentiated stromal tumours. Occasionally, older children may also have Sertoli cell tumours that behave in a malignant manner [42].

In another report of 51 patients with prepubertal testicular tumours, 3 had a stromal neoplasm. The histology was suggestive of Leydig cell tumour in all. All presented with precocious puberty and elevated serum testosterone and androstenedione concentrations and underwent radical inguinal orchiectomy for stage I disease. No recurrences were noted.

Clinical Presentation

Nearly all testicular stromal tumour patients present with a painless mass. The mass is usually easily palpable and visible on ultrasound and may have a heterogeneous appearance. Patients may have gynaecomastia or signs of precocious puberty. Patients with testicular juvenile granulosa cell tumours often present in the first 6 months of life and usually lack hormone-related symptoms. Children with Leydig cell tumours may present with precocious puberty or gynaecomastia and increased 17-KS.

When the diagnosis of a testicular tumour is considered, αFP measurement is essential and inhibin measurement should be considered. In patients near or after puberty, βhCG concentration should also be measured. Testicular granulosa cell tumours may secrete inhibin but the use of inhibin as a tumour marker has not been established. Immunohistochemical staining of testicular stromal tumours has been well described. Inhibin A staining has been found to distinguish testicular SCTs from GCTs. Oestrogen and progesterone receptors may also be present in up to 39% of malignant Leydig cell tumours [43].

Clinical Genetics

Testicular juvenile granulosa cell tumours may be seen in association with structural abnormalities of the Y chromosome or in patients with DSD. In addition, Carney

syndrome may present with cutaneous lentiginosis, atrial myxoma, Cushing syndrome, acromegaly and/or papillary and FTCs in addition to gonadal stromal tumours. PJS is characterized by cutaneous lentiginosis, intestinal polyps and a predisposition to gastrointestinal and extraintestinal malignancies. Both may be associated with large cell calcifying Sertoli cell tumours. Carney syndrome has also been associated with unilateral or bilateral Leydig cell tumours. In both PJS and Carney syndrome, the lesions are often hormone producing and may cause gynaecomastia in children or adults.

Management

On the basis of their individual management strategy, each testicular tumour will be considered separately.

Testicular juvenile granulosa cell tumours present in infancy, usually in children under the age of 6 months. By immunohistochemistry, they are characteristically positive for inhibin and are considered benign, with little to no evidence of recurrence or metastases. Testis-sparing surgery is generally recommended if a reasonable amount of normal testicular tissue remains. No adjuvant therapy is recommended for patients with stage I testicular juvenile granulosa cell tumours. In these tumours, serum αFP is expected to be within the normal range for age, although there is a wide physiological range in infancy that may make this determination challenging. Surgical resection is curative for testicular juvenile granulosa cell tumour.

Sertoli cell tumours are considered generally benign in children <5 years of age, and radical orchiectomy alone is sufficient. In older children, evaluation to rule out metastatic disease with CT of the chest, abdomen and pelvis is essential. In case of metastatic disease, aggressive surgical and adjuvant therapy is recommended.

Large cell calcifying Sertoli tumours tend to follow a more benign course than other Sertoli cell tumours in older children and adolescents. The large cell calcifying Sertoli tumour seen in Carney syndrome is unlikely to metastasize and orchidectomy is likely to be curative. Testis-sparing surgery has also been described in this group [44].

Leydig cell tumours are seen in prepubertal males and carry a favourable prognosis. Orchidectomy or testis-sparing surgery alone is likely to be curative. Hormonal symptoms may be associated with elevation of 17-KS and may require the consultation of a pediatric endocrinologist. Testicular Sertoli–Leydig cell tumours are quite rare. Finally, undifferentiated stromal tumours harbour malignant potential and prepubertal and post-pubertal males with these tumours should undergo a metastatic evaluation.

The Prepubertal Testicular Tumor Registry described 43 patients registered with stromal tumours. Ten had an unspecified stromal tumour and one developed metastases. The remaining 32 included 10 Sertoli cell tumours, 5 Leydig cell tumours, 9 juvenile granulosa cell tumours and 8 mixed/undifferentiated stromal tumours. The single patient diagnosed with metastatic mixed/undifferentiated stromal tumour died. None of the patients with Sertoli, Leydig or juvenile granulosa cell tumour had metastases at diagnosis but four cases of Sertoli cell tumours with malignant behaviour in prepubertal males have been reported in the literature [31].

The staging and adjuvant chemotherapy recommendations follow the same principles as GCTs.

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13

Endocrine Late Effects of Cancer Treatments

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KEY LEARNING POINTS

- Endocrine late effects are observed in up to 50% of childhood cancer survivors (CCS).
- Injury to endocrine organs may result from tumour growth or surgery, direct or scatter radiotherapy and/or treatment with alkylating agent chemotherapy.
- Endocrine organ impairment in CCS may involve the hypothalamus/pituitary, the thyroid and the gonads, as well as the systems regulating bone health and metabolism.
- The risk of developing a particular endocrine late effect varies according to tumour location, treatment modality and a variety of host factors.
- A systematic risk-based screening approach that facilitates the early diagnosis and treatment of vulnerable CCS may improve long-term overall health outcomes.

The development of curative therapy for childhood cancer has been a resounding success. In the USA, 5 year survival rates for children aged 10–14 years exceed 80% for all cancers. The survival rate following childhood acute lymphoblastic leukaemia (ALL), the most common malignancy in childhood, improved from 20% in 1961 to 93.5% in 2009. Since cancer mortality for children and adolescents has declined over the last four decades, a growing number of individuals treated for cancer during childhood survive into middle age. This has resulted in an estimated 1 in 530 young adults aged 20–39 being a CCS [1].

Successful treatment relies on combinations of surgery, multi-agent chemotherapy and radiotherapy as well as on supportive care. Cancer treatments may also have long-lasting detrimental effects on various organ systems, including the endocrine glands, which may appear years, sometimes decades, after completion of cancer therapy and are referred to as treatment-related late effects. Endocrine complications are among the most commonly reported late effects with one out of two survivors experiencing at least one hormonal or reproductive disorder during his or her lifetime [2].

The delayed appearance of treatment-related late effects and the possible contribution of undiagnosed and/or untreated dysfunction to poor overall health outcomes may challenge the delivery of adequate care to CCS [3]. A risk-based screening approach where patients are evaluated for a given condition at defined intervals using specific diagnostic tools based on their cancer and treatment history has become standard in survivor care. Long-term follow-up guidelines have been proposed by consortia in North America (Children's Oncology Group [COG], www.survivorshipguidelines.org), the UK (Children's Cancer and Leukaemia Group, http://www.cclg.org.uk/dynamic_files/LTFU-full.pdf), Scotland (Scottish Intercollegiate Guidelines Network, http://sign.ac.uk/pdf/sign132.pdf) and the Netherlands (Dutch Children's Oncology Group); efforts are presently underway to harmonize the recommendations of these groups.

We present an overview of the most common endocrine late effects in CCS (Tables 13.1 and 13.2) but the continual changes in cancer treatments require continuous reappraisal. Information based on older cancer treatment regimens remains relevant to the provision of

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Companion website: www.wiley.com/go/dattani/brookcpe7

Table 13.1 Endocrine late effects of cancer treatments: hypothalamo–pituitary dysfunction.

	GH deficiency (pediatric) μ	Central precocious puberty	Hyperprolactinaemia	TSH deficiency	LH/FSH deficiency	ACTH deficiency
1-Population at risk						
Tumour- and treatment-related risk factors						
Tumour factors	Hypothalamo–pituitary location or surgery	Hypothalamic/thalamic/optic pathway tumours – NF-1 Hydrocephalus	Hypothalamo–pituitary location or surgery	Hypothalamo–pituitary location or surgery	Hypothalamo–pituitary location or surgery	Hypothalamo–pituitary location or surgery
Radiotherapy[a]	≥18 Gy	≥18 Gy	≥40 Gy	≥30 Gy	≥30 Gy	≥30 Gy
Total body irradiation (TBI)	≥10 Gy 1 fraction ≥12 Gy multiple fractions	Not if delivered alone	Not if delivered alone	Not if delivered alone	Not if delivered alone	Not if delivered alone
Host factors						
Young age at diagnosis	Yes	Yes	No	No	No	No
Female sex	No	Yes	No	No	No	No
2-Surveillance modality						
History	Nutritional status, notable pubertal or growth changes	Nutritional status, notable pubertal or growth changes	Pubertal changes, menstrual history galactorrhoea	Symptoms of hypothyroidism, slow growth	Pubertal changes, menstrual history, stamina	Fatigue, weight loss, infections, malaise, dizziness
Physical	Height, sitting height,[b] growth velocity, pubertal staging, weight, BMI	Height, sitting height,[b] growth velocity, pubertal staging, weight, BMI	Height, pubertal staging, weight, BMI	Height, growth velocity, pubertal stage, weight, BMI, hair/skin, neck palpation	Height, growth velocity, pubertal staging, weight, BMI	Height, weight, BMI, blood pressure
Laboratory screening	None systematically[c]	None systematically[c]	Prolactin	TSH,[d] free T4	LH, FSH, morning testosterone (males) or oestradiol (females)	8 AM cortisol
3-Frequency of screening						
Minimal frequency – more frequent assessment to be performed as clinically indicated	Every 6 months until growth is completed	Every 6 months until normal age for puberty	Yearly	Every 6 months until growth is completed then yearly	Baseline at 14 years (males) or 13 years (females), then at least yearly	Yearly

4-Confirmatory or additional testing if screening is positive						
Laboratory	GH stimulation test	LH, testosterone or oestradiol	None	None	Repeat morning testosterone	Low-dose ACTH test
		GnRH or GnRH agonist testing	None	None	Reassess male obese patients after weight loss	
Diagnostic imaging	Bone age X-ray	Bone age X-ray, pelvic ultrasound (girls)[e]	CNS imaging if indicated	None	None	None
5-Treatment						
Modality	GH replacement	GnRH agonists	Dopaminergic agonists	Levothyroxine	Sex hormone replacement	Hydrocortisone
Special considerations	Caution in regard to superimposed precocious puberty	Caution in regard to superimposed GH deficiency	Not useful in patients with primary hypogonadism	Diagnose and treat ACTH deficiency first	Refer to reproductive endocrinologist as needed	Stress dose teaching

μ, guidelines for the screening and management of GH deficiency after final height attainment and in adult survivors of childhood cancer are in development.
[a] Includes radiation to the following areas: cranial ears/infra-temporal, nasopharyngeal, orbital/eyes and Waldeyer's ring.
[b] In patients with spinal exposures to radiotherapy such as TBI or cranio-spinal radiation.
[c] Testicular volume is not a reliable indicator of pubertal development in males exposed to gonadotoxic chemotherapy or testicular radiation – LH and testosterone measurements should be considered if abnormal puberty timing is suspected in these patients.
[d] TSH measurement is useful for screening but should not be used in the follow-up of patients with known TSH deficiency. Screening recommendations were adapted from the Children's Oncology Group Long-Term Follow-Up Guidelines Version 4.0 (www.survivorshipguidelines.org).
[e] Consider for measurement of uterine length and ovarian volumes in girls with discrepant clinical and laboratory features.

Table 13.2 Endocrine late effects of cancer treatments: common primary thyroid, gonadal, bone and metabolic disorders.

	Primary hypothyroidism	Thyroid cancer	Leydig cell failure	Male germ cell failure	Premature ovarian insufficiency	Decreased bone mineral density	Overweight, obesity, glucose intolerance
1-Population at risk							
Tumour- and treatment-related risk factors							
Tumour or surgical factors	Thyroidectomy	If part of cancer predisposition syndrome	Bilateral orchiectomy	Bilateral orchiectomy	Bilateral oophorectomy	Leukaemia	Hypothalamic tumours or surgery
Radiotherapy dose to organ	≥10 Gy[a]	Risk increases with dose up to 30 Gy and then decreases[a]	≥20 Gy[b]	Possible ≥0.15 Gy[b] High risk ≥2 Gy[b]	>0 Gy[b]	Cranial/spinal total body	Cranial total body abdominal
Haematopoietic stem cell transplant	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Chemotherapy	Not if delivered alone[c]	Not if delivered alone	Alkylating agents	Gonadotoxic agents[d]	Gonadotoxic agents[d]	Glucocorticoids	Glucocorticoids
Others	Radioactive iodine ^{131}I-MIBG	^{131}I-MIBG	—	—	131I-MIBG	Methotrexate cyclosporine, tacrolimus	GvHD
Host factors							
Age at diagnosis with most risk	Older	Younger	No association	No association	Older	Younger	Varies
Female sex	Yes	Yes	Not applicable	Not applicable	Not applicable	No	Yes
2-Surveillance modality							
History	Hypothyroidism signs, slow growth	Thyroid nodule or cervical lymph node	Pubertal changes, stamina	Fertility concerns (adult males)	Pubertal changes, menstrual history, stamina	Nutrition, puberty, fractures, medications	Nutrition, physical activity
Physical	Height, growth velocity, pubertal stage, weight, BMI, the hair/skin, the neck	Palpation of the neck by experienced provider	Height, growth velocity, pubertal staging, weight, BMI	Genital examination, testicular volume	Height, growth velocity, pubertal staging, weight, BMI	Height, growth velocity, pubertal staging, weight, BMI	Height, pubertal staging, weight, BMI, waist to height ratio
Laboratory or imaging screening	TSH, free T4	No consensus regarding ultrasound	LH, FSH, morning testosterone	FSH, AM testosterone, semen analysis	FSH, oestradiol	Bone mineral density (DXA or qCT)	Fasting glucose and lipids, HbA1c

3-Frequency of screening							
Minimal frequency – more frequent assessment to be performed as clinically indicated	Every 6 months until growth is completed and then yearly	Yearly	Baseline at 14 years and then at least yearly	As requested by adult patients	Baseline at 13 years and then at least yearly	At entry into long-term follow-up and then as clinically indicated	Yearly[e]
4-Confirmatory or additional testing if screening is positive							
Laboratory	None	Ultrasound-guided fine needle aspiration	Repeat morning testosterone	None	None	Vitamin D 25 level	Oral glucose tolerance test
Diagnostic imaging	None	Neck ultrasound	None	None	None	None	None
5-Treatment							
Modality	Levothyroxine	Surgery ± ablation radioactive iodine	Sex hormone replacement	Sperm banking (prevention)	Sex hormone replacement mature oocyte cryopreservation	Vitamin D supplement, physical activity, dietary intervention	Diet and lifestyle modification
Special considerations	Diagnose and treat ACTH deficiency first	Total thyroidectomy preferred	Refer to reproductive endocrinologist as needed	Refer to reproductive endocrinologist as needed	Refer to reproductive endocrinologist as needed	Treat-associated endocrinopathies	Pharmacotherapy as indicated

DXA, dual X-ray absorptiometry; qCT, quantitative computed tomography. Screening recommendations were adapted from the Children's Oncology Group Long-Term Follow-Up Guidelines Version 4.0 (www.survivorshipguidelines.org).

[a] Radiation fields potentially affecting the thyroid include cervical (neck), spinal (neck or whole), supraclavicular, cranial, nasopharyngeal, oropharyngeal, Waldeyer's ring, subtotal lymphoid irradiation, total lymphoid irradiation (TLI), mantle (including mini or extended fields), mediastinal and TBI.

[b] Radiation fields potentially affecting the gonads include the flank/hemi-abdomen, whole abdomen, inverted Y, pelvic, bladder, iliac, TLI, TBI, whole/lumbar/sacral spine (females), vaginal (females), prostate (males), inguinal (males), femoral (males) and scrotal/testicular (males).

[c] Table does not include novel targeted therapies such as tyrosine kinase inhibitors or immunomodulators.

[d] See Table 13.3.

[e] Labs suggested every 2 years.

care and counselling to CCS treated decades ago. We conclude with a section dedicated to the endocrine complications associated with some of the more novel approaches to cancer treatment, such as chemotherapy agents targeting tumour growth pathways and proton beam radiotherapy.

Prevalence and Risk Factors for Endocrine Late Effects

Survivors of Childhood Central Nervous System (CNS) Tumours

Survivors of childhood CNS tumours commonly experience hypothalamo–pituitary axis (HPA) dysfunction, particularly with tumours developing near the hypothalamus or the pituitary gland and those treated with cranial radiotherapy (CRT). Primary thyroid and gonadal dysfunctions are known sequelae of direct or scatter exposure following cranio-spinal irradiation (CSI). Gonadal germ cell injury may also be caused by exposure to alkylating agent chemotherapy (Table 13.3).

Table 13.3 Chemotherapy agents associated with potential gonadal toxicity.

Category	Drug
Alkylating agents	Busulfan
	Carmustine (BCNU)
	Chlorambucil
	Cyclophosphamide
	Ifosfamide
	Lomustine (CCNU)
	Mechlorethamine
	Melphalan
	Procarbazine
	Thiotepa
Heavy metals	Carboplatin
	Cisplatin
Non-classical alkylators	Dacarbazine
	Temozolomide

Source: The Children's Oncology Group long-term follow-up guidelines version 4.0 – October 2013 (www.survivorshipguidelines.org).

Growth Failure and HPA Dysfunction

When related to direct anatomical damage or following surgical resection, HPA dysfunction generally presents at or soon after diagnosis of a CNS tumour and tends to involve multiple hormonal systems from the anterior and/or posterior pituitary. By contrast, radiation-induced HPA dysfunction often presents several months or years after CRT, does not involve the posterior pituitary and may not encompass all HPA hormones simultaneously since the dose thresholds vary for the different anterior pituitary hormones [3, 4]. In a study of 748 long-term adult survivors exposed to CRT during childhood and followed for a mean of 27.3 years, the prevalence of at least one and more than one anterior pituitary deficiency was 51.4 and 10.9%, respectively (Figure 13.1) [3]. The nature (neurovascular damage versus direct neuronal injury) and site (hypothalamic versus pituitary) of radiation-induced damage have not been established. Evidence supporting a hypothalamic rather than pituitary origin includes the observation of hyperprolactinaemia, attributed to the loss of dopaminergic inhibition by the hypothalamus following radiation doses >50 Gy, and a preserved growth hormone (GH) response to exogenous GH–releasing hormone (GHRH) in individuals exposed to CRT [5]. Hypothalamic factors have been hypothesized to exert a trophic effect on pituitary hormone-producing cells, which may explain the differential sensitivity to radiation among hypothalamo-pituitary functions [6]. Hypothalamo-pituitary dysfunction relates to radiotherapy in a dose- and time-dependent fashion; the higher the dose of radiation and the longer the time since treatment, the higher the risk [3, 4]. Hence, radiation-induced HPA dysfunction may occur years or even decades after the completion of therapy and at-risk patients should be offered lifelong monitoring (Table 13.1).

Growth Hormone Deficiency

Growth hormone deficiency (GHD) is the most frequent and often the only radiation-induced HPA dysfunction in CCS (Figure 13.1) [3]. In a study of 88 survivors of childhood medulloblastoma and other embryonal tumours treated with CRT, CSI and high-dose myeloablative chemotherapy followed by autologous stem cell rescue, the cumulative incidence of GHD was 93 ± 4% 4 years after the initial cancer diagnosis [4]. The risk of radiation-induced GHD increases with the dose of radiotherapy and the length of follow-up. Younger age at treatment with CRT has inconsistently been reported as a significant risk factor of GHD [3].

Central Precocious Puberty

Tumours located near the hypothalamus and optic pathways, such as low-grade gliomas, often in the context of neurofibromatosis type 1 (NF-1), and the exposure of this region to radiotherapy (doses 18–50 Gy) are risk factors for central or gonadotropin-dependent precocious puberty (CPP). The overall prevalence in a cohort of 500 at-risk childhood CNS tumour survivors assessed prospectively was 15.2% [7]. CPP appeared on average 3.5 ± 2.4 years after the diagnosis of a CNS tumour in this study [7]. CPP is therefore among the most common HPA dysfunctions in this population [3, 7]. The prevalence

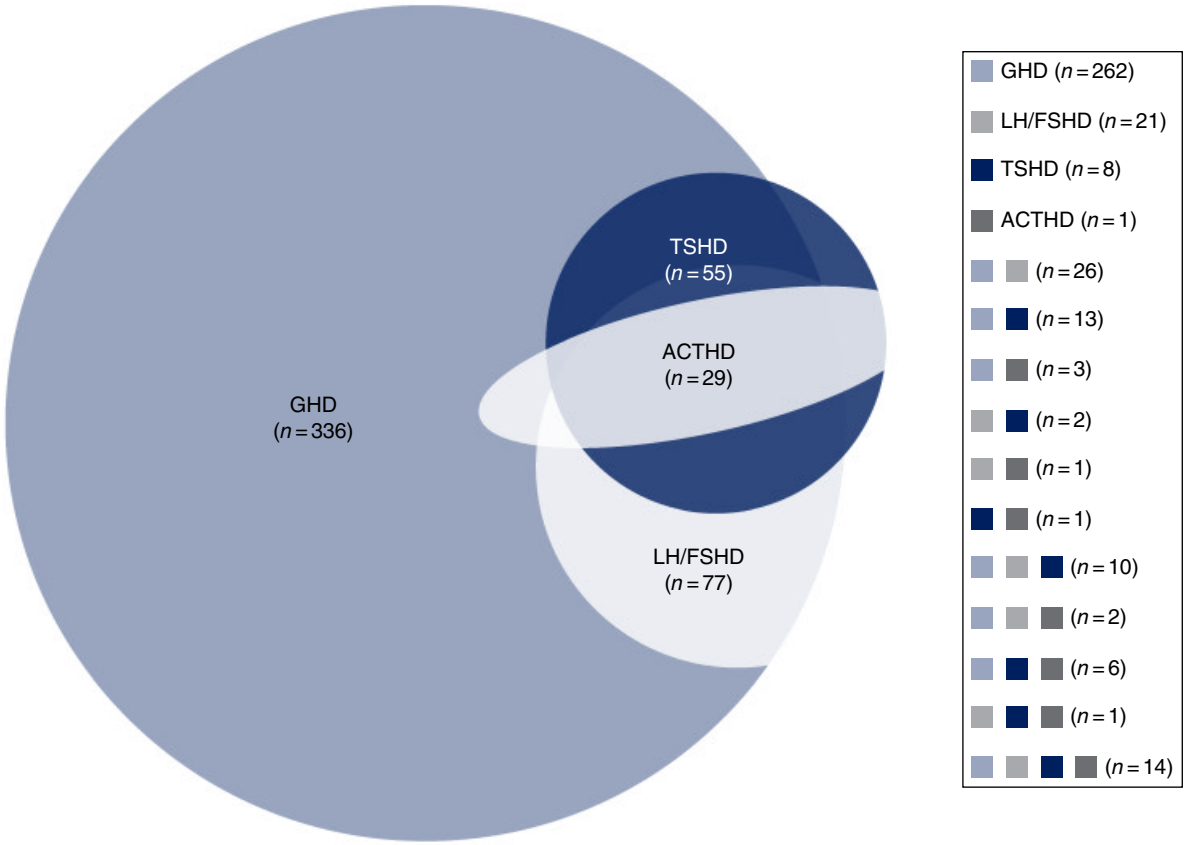

Figure 13.1 Relative proportions and overlap among anterior pituitary deficiencies following CRT [3].

was substantially higher when CPP resulted from tumour location (29.2%) than when it occurred in relation to CRT (6.6%) [7]. Additional risk factors for CPP include hydrocephalus, female sex, CRT before the age of 5 years and increased BMI.

Hypogonadotropic Hypogonadism

Patients with hypogonadotropic hypogonadism (LH/FSH deficiency [LH/FSHD]) experience low sex hormone production because of inadequate hypothalamo–pituitary stimulation. Depending on the age of onset, LH/FSHD may manifest as pubertal delay, interrupted puberty or adult symptoms of sex hormone deprivation. The reported prevalence of LH/FSHD ranges from 5 to 10% in CCS treated with CRT and has been associated with doses of radiation ≥30 Gy [3, 5]. Interestingly, patients with a history of CPP may paradoxically experience LH/FSHD as a radiation-induced late effect years later and require sex hormone replacement therapy [7].

Central Hypothyroidism

The prevalence of central hypothyroidism (TSH deficiency [TSHD]) in CCS varies between 3.4 and 7.5% [3, 4]. The 4-year cumulative incidence of TSHD reached 28 ± 8% in a study of long-term endocrine outcomes among childhood embryonal (e.g. medulloblastoma) CNS tumour survivors exposed to CRT and CSI [4]. Exposure to doses of CRT ≥30 Gy represents the main risk factor for TSHD as a late effect [3, 5].

Central Adrenal Insufficiency

Patients with central adrenal insufficiency (adrenocorticotropic hormone deficiency [ACTHD]) have insufficient cortisol secretion because of inadequate hypothalamo–pituitary stimulation. The reported prevalence of ACTHD in CCS ranges between 4 and 43% due to differences in patient populations and testing modalities [3]. The cumulative prevalence of ACTHD was 38 + 6% in the study of children with embryonal CNS tumours treated with CRT and CSI [4]. Exposure to doses of CRT ≥30 Gy is the main risk factor for ACTHD as a late effect [3, 5].

Hyperprolactinaemia

Hyperprolactinaemia may result from the loss of hypothalamic inhibition on prolactin secretion following doses of CRT >30–50 Gy [5]. Hyperprolactinaemia is usually asymptomatic and rarely warrants treatment.

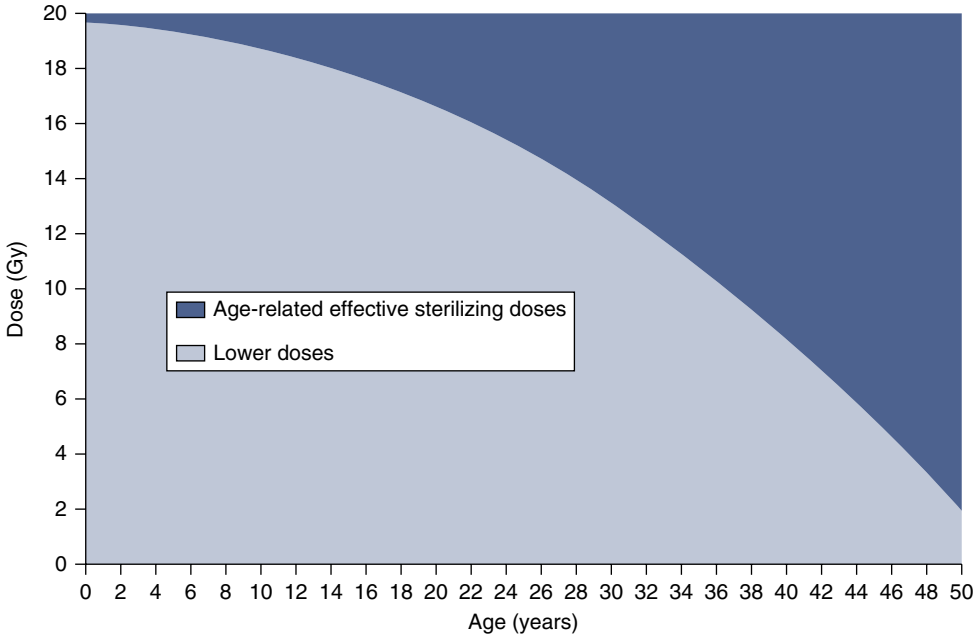

Figure 13.2 Effective sterilizing dose for age at treatment and premature ovarian insufficiency prediction according to age and radiation dose [10]. *Source:* Reproduced with permission of Elsevier.

Thyroid Disorders

The thyroid gland may be exposed to scatter radiation after CSI or whole-brain CRT. Individuals who underwent radiation to these treatment fields have a higher likelihood of thyroid disorders, especially primary hypothyroidism and thyroid cancer. The cumulative incidence of primary hypothyroidism was 65 ± 7% by 4 years in the cohort of patients with CNS embryonal tumours treated with CRT and CSI [4]. Hyperthyroidism has been reported in survivors of childhood CNS tumours following CSI but tends to be transient given the overriding frequency of primary hypothyroidism as a late effect. Treatment with CSI at a young age is a known risk factor for thyroid cancer, which was the most commonly reported second malignancy outside the CNS among 455 survivors of childhood medulloblastoma, occurring after a mean latency period of 25 years [8].

Disorders of the Gonads

Males

The main risk factors for primary testicular dysfunction in survivors of childhood CNS tumours are exposure to alkylating agent chemotherapy regimens (Table 13.3) and scatter radiation from CSI. Leydig cell function may be impaired following the exposure to high doses of alkylating agents but does not seem to be affected by scatter radiation from CSI [9]. Germ-cell function is more vulnerable to alkylating agents and scatter radiotherapy and more likely to be impaired in CCS treated with these modalities than Leydig cell function.

Females

Ovaries are vulnerable to damage from both chemotherapy (Table 13.3) and scatter radiation related to CSI (Figures 13.2 and 13.3). The prevalence of primary ovarian insufficiency is estimated at 26% in young survivors of childhood medulloblastoma; this is probably an underestimate given that patients may experience premature menopause in subsequent years and with longer follow-up [12].

Bone mineral Density (BMD) Deficit

Childhood CNS tumour survivors are at risk of developing bone mineral density (BMD) deficits. Multiple factors may contribute to this risk, including toxicity from treatment (glucocorticoids, CRT, CSI), hormonal deficiencies (GHD and hypogonadism) and decreased physical activity.

Overweight and Obesity

Rates of obesity and overweight in the overall population of childhood CNS tumour survivors are similar to those observed in the general population but patients with hypothalamo-pituitary injury, especially due to damage from tumour and/or surgery, have a significantly higher risk of obesity and may experience particularly severe 'hypothalamic' or 'central' forms of this condition. Up to 55% of patients with a craniopharyngioma experience hypothalamic obesity despite the replacement of all hormonal deficits [13]. Contemporary treatment strategies for craniopharyngioma have moved away from total

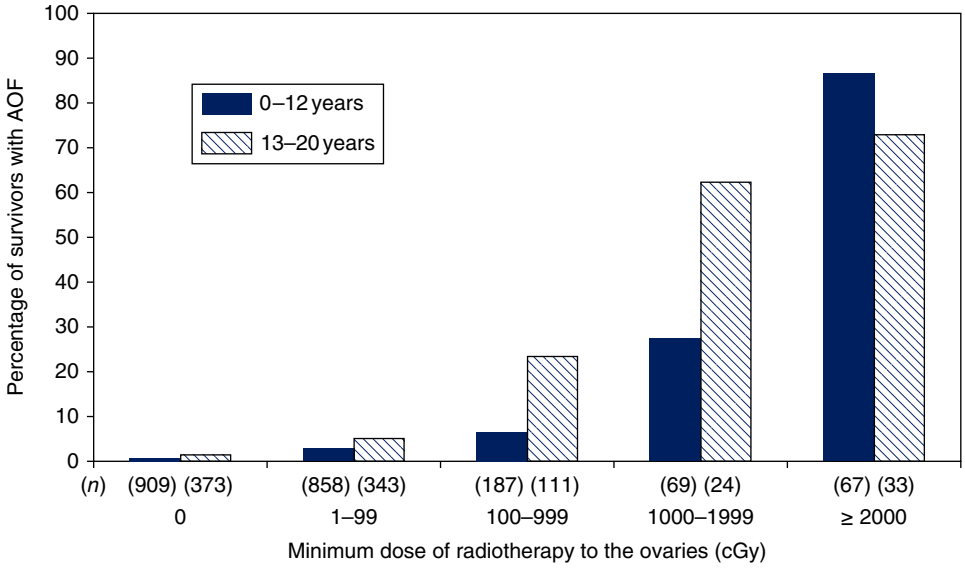

Figure 13.3 Percentage of subjects with acute ovarian failure (no spontaneous resumption of puberty or menses after completion of therapy) according to age at diagnosis of 0–12 years (solid bar) and 13–20 years (striped bar) versus dose of radiation to the ovary [11]. *Source:* Reproduced with permission of Oxford University Press.

resection; the integration of high precision radiotherapy, more recently via proton beam technology, with hypothalamus-sparing surgery, has resulted in excellent long-term progression-free survival [13]. These novel approaches will hopefully limit perioperative hypothalamic damage and improve metabolic and overall long-term health outcomes.

Survivors Treated with Haematopoietic Stem Cell Transplantation (HSCT) for Haematological Malignancies

Patients are treated with HSCT for a variety of cancers, non-malignant haematological disorders, such as Fanconi anaemia, and inborn errors of metabolism. In *autologous* HSCT, normal haematopoietic stem cells are harvested from the patient and reinfused into the same individual after completion of myeloablative conditioning therapy. Patients treated with *allogeneic* HSCT are infused with healthy haematopoietic stem cells harvested from another individual, a related or unrelated ideally HLA-matched donor in order to replace malignant or abnormal stem cells. Allogeneic HSCT requires the prior conditioning of the transplant recipient with myeloablative treatments using high-dose chemotherapy with or without total body irradiation (TBI). Patients successfully treated with HSCT may develop long-term complications not only as a result of exposure to chemotherapy and TBI but also (for allogeneic HSCT recipients) of graft versus host disease (GvHD) and its treatments. Given the vulnerability of the endocrine organs to radiotherapy and chemotherapy, endocrine sequelae are among the most prevalent late effects following HSCT. In a study of long-term outcomes after successful allogeneic HSCT, 59% of individuals treated before the age of 10 years had at least one hormonal dysfunction [14].

Growth Failure and HPA Dysfunction

Impaired growth has been reported in 20–84% of pediatric HSCT recipients. Many factors contribute to impaired growth following HSCT including GvHD, chronic illness, undernutrition, radiation-induced damage to the skeletal growth plates, GHD, hypothyroidism and pubertal disorders. Pediatric HSCT survivors may lose 1–2 SD in height measurements between the time of HSCT and final height attainment. Treatment with TBI, previous exposure to CRT, young age at HSCT and male sex are risk factors for impairment. The loss in growth potential worsens during puberty and affects sitting height disproportionately. These observations indicate that radiation-induced damage to the skeletal growth plates plays an important role in a subset of patients whose ability to experience a normal pubertal growth spurt is compromised even in the absence of GHD.

The contribution of GHD to growth impairment following HSCT is difficult to establish given the multiplicity and significance of other risk factors. In keeping with the vulnerability of the HPA to radiation, the main risk factor for GHD is TBI. The estimated prevalence of GHD in patients treated with TBI varies between 20 and 40%. Doses <8 Gy are less likely to be associated with GHD while younger age at HSCT and single-fraction regimens

may be associated with a higher risk [15]. Recipients of pediatric HSCT conditioned with TBI in the absence of prior exposure to CRT have a very low risk of developing hypothalamo-pituitary dysfunction other than GHD. The prevalence of TSHD and ACTHD in CCS treated with HSCT was probably overestimated in studies where complex dynamic testing modalities, such as nocturnal TSH surge and 11-deoxycortisol response to metyrapone, respectively, were used rather than more commonly used tests.

Thyroid Disorders

Thyroid disorders following HSCT include primary hypothyroidism, autoimmune disease and neoplasms. The reported prevalence of primary hypothyroidism ranges between 14 and 52% following HSCT. The main risk factor is exposure to TBI, with a higher risk in patients treated with single-fraction radiotherapy. Patients conditioned for HSCT with chemotherapy alone using busulfan and cyclophosphamide may experience compensated and often transient forms of primary hypothyroidism. Younger age and longer duration of follow-up have been associated with a higher risk of primary hypothyroidism following HSCT. Autoimmune thyroid disease has been observed following HSCT in a small number of patients manifesting as hypo- or hyperthyroidism. The acquisition of abnormal clones of donor T- or B-lymphocyte cells is postulated as the most likely explanation for this phenomenon. Hyperthyroidism in this context is often transient and may be followed by primary hypothyroidism; only a minority of patients requires treatment with antithyroid medications or radioactive iodine [16]. Patients treated with TBI have an increased risk of developing secondary thyroid cancer. Young age at transplant, a history of chronic GvHD and female sex are additional risk factors for thyroid cancer in this population [17].

Disorders of the Gonads

Males

The main risk factors for primary testicular dysfunction in CCS treated with HSCT are treatment with alkylating agent chemotherapy (Table 13.3) and/or TBI. Leydig cell function is generally unaffected by standard doses of cyclophosphamide or by TBI as long as the cumulative dose of radiation to the testes is <20 Gy. In contrast to Leydig cell function, spermatogenesis is frequently impaired in CCS treated with HSCT. The combination of cyclophosphamide (especially at cumulative doses >200 mg/kg) and busulfan has been associated with impaired germ cell function [18]. Young age at treatment, absence of chronic GvHD and longer duration of follow-up have been associated with better chances of retaining some germ cell function, whereas only a few men treated with TBI have reported fathering children [18]. Testicular shielding may help preserve testicular function in patients treated with TBI; sperm banking should nevertheless be offered to all sexually mature males capable of producing a semen sample before they undergo HSCT.

Females

Female CCS treated with HSCT have a high risk of developing premature ovarian insufficiency, which has been reported in up to 84% of patients. Most patients conditioned for HSCT with cyclophosphamide and busulfan require long-term sex hormone replacement therapy. The impact of TBI on ovarian function seems to depend primarily on the patient's age at the time of treatment (Figures 13.2 and 13.3). Up to 50% of girls treated with TBI at prepubertal ages may experience a spontaneous onset of pubertal development and attain menarche at a normal age. By contrast, premature ovarian insufficiency is almost universal in female CCS exposed to TBI after 10 years of age [19]. Ovarian shielding may help preserve function in patients treated with TBI; long-term data demonstrating outcomes after this practice are very limited. High rates of miscarriage have been reported in women with a history of treatment with TBI, attributed to the adverse repercussions of TBI on uterine volume and/or blood supply [20].

Bone Mineral Density Deficit

Patients treated with allogeneic HSCT have an increased risk of BMD deficit as a result of disease-related impairments, treatment toxicities and transplant complications. The prevalence of moderate (BMD Z score between −1 and 1.9 adjusted for age and sex) and severe (Z score ≤−2) BMD deficit was 18 and 16% at baseline and increased to 33 and 19%, respectively, 1 year after HSCT in a prospective study of 49 pediatric HSCT recipients assessed using dual X-ray absorptiometry (DXA) [21]. Risk factors for BMD deficit include young age at HSCT, treatment with TBI and prior CRT, the latter probably because of GHD and/or sex hormone deficiency. The pathophysiology of persistent BMD deficit following HSCT is not well understood. Impaired differentiation of mesenchymal stem cells into osteoblasts and their preferential rearing towards adipogenesis (instead of osteogenesis), as evidenced by increased marrow and visceral adiposity, have been suggested as potential mechanisms [22]. Advances in understanding the pathophysiology of this disorder, which seems singularly intertwined with post-HSCT metabolic complications, may set the stage for more specific, targeted interventions in the future.

Insulin Resistance and Diabetes Mellitus

Patients who have undergone pediatric HSCT develop insulin insensitivity, glucose intolerance and/or various

other components of the metabolic syndrome such as hypertension and dyslipidemia at higher than expected rates. The prevalence of diabetes mellitus in patients treated with HSCT during childhood varies between 4 and 17%. Insulin insensitivity seems to be more prevalent and persistent, potentially affecting up to 52% of assessed individuals long term. Treatment with TBI is the principal risk factor for insulin insensitivity and/or diabetes mellitus following HSCT. These complications do not seem to be related to body mass index (BMI); their pathophysiology may involve changes in body fat distribution and/or a certain degree of pancreatic beta-cell injury [23].

Endocrine Late Effects in Survivors of Non-HSCT Requiring Childhood Leukaemia

ALL accounts for ~25% of childhood cancers. Advances in the therapy and risk stratification have substantially improved survival rates and decreased treatment toxicity over the past 5 decades. Intrathecal chemotherapy administration and intensified systemic chemotherapy have resulted in a marked decline in the use of prophylactic CNS irradiation, a major cause of endocrine late effects, since the 1980s. Acute myeloblastic leukaemia (AML) is less common in children than ALL; progress in its cure has followed the general trend of improvement among childhood cancers with a survival rate presently exceeding 70% albeit with a frequent need for HSCT.

Growth Failure and HPA Dysfunction

Patients surviving childhood leukaemia, including those treated with chemotherapy alone, may not fully recover their pre-diagnosis growth potential. While childhood-onset GHD is a determining factor for short stature in survivors of ALL treated with CRT, a moderate loss in height potential (–0.59 SD ± 0.86), irrespective of GH status, has been described in ALL survivors treated with modern chemotherapy-only regimens [24]. Treatment-related changes in pubertal timing and possible direct toxicity to the growth plates are among the non-GHD factors, contributing to growth failure in the absence of CRT or CSI. Central precocious puberty (CPP) has been described following CRT for childhood leukaemia [7]. Given the dose- and time-dependent relationship with CRT, childhood leukaemia survivors who were treated with prophylactic CRT during childhood have an increased risk of developing adult-onset GHD and LH/FSHD and, to a lesser extent, TSHD and ACTHD throughout adulthood and middle age [3].

Disorders of the Thyroid

Patients surviving childhood ALL not treated with HSCT may subsequently develop thyroid disease because of exposure to CSI and/or CRT. Prophylactic CRT (without CSI or TBI) for childhood ALL is not associated with a substantial risk of primary hypothyroidism. Papillary thyroid carcinoma has been reported in a small number of patients treated with prophylactic CRT for ALL as a result of scatter radiation to the thyroid. It is notable that patients treated with this modality several decades ago did not benefit from the significant improvements in the precision of the delivery of radiotherapy that occurred over the years.

Disorders of the Gonads

Males

Males treated for childhood leukaemia have a higher than expected risk of developing both Leydig cell and germ cell failure following alkylating agent chemotherapy (Table 13.3) and direct testicular radiotherapy, which is used to treat testicular relapse. Prevalence estimates for Leydig cell dysfunction after alkylating agent chemotherapy range between 10 and 57%. In this context, however, Leydig cell dysfunction is generally subclinical; the majority of affected patients have normal plasma testosterone concentrations with an elevated LH. Patients treated with doses of radiation >20 Gy to the testes have the highest risk of developing Leydig cell failure, with more than 80% requiring testosterone replacement during childhood and adolescence. Germ cells have an even higher degree of vulnerability to cancer treatments than Leydig cells. Treatment with alkylating agent chemotherapy at cumulative cyclophosphamide equivalent doses ≥4000 mg/m^2 is associated with impaired spermatogenesis [25]. Impaired semen production appears at doses of testicular irradiation as low as 0.15 Gy and recovery of spermatogenesis following 2–3 Gy is unlikely.

Females

Despite the vulnerability of the ovaries to alkylating agent chemotherapy, females treated with standard chemotherapy doses for childhood leukaemia are generally able to enter puberty normally and experience regular menses thereafter. Data on the long-term reproductive outcomes of female childhood leukaemia survivors who were treated with contemporary protocols remain limited. Further research is needed to truly understand the reproductive risk for this population given the risk of premature menopause [12].

Bone Mineral Density Deficit

Childhood leukaemia is associated with significant short- to medium-term bone morbidity. Radiological abnormalities of the skeleton, including osteopenia and fractures, have been noted in up to 70% of children at diagnosis as a result of the disease process. Acute toxicity from chemotherapy, most notably due to the prolonged exposure to

high doses of systemic glucocorticoids, leads to additional complications including non-traumatic vertebral fractures and osteonecrosis. Data from prospective and longitudinal assessments indicate that recovery of BMD starts shortly after completion of therapy for ALL [26]. BMD deficits may nevertheless persist in the medium to long term depending on the severity of the impairment at baseline, the time necessary for healing, the presence of co-morbidities and suboptimal lifestyle choices.

Overweight, Obesity and Glucose Intolerance

Overweight and obesity are common findings in survivors of childhood ALL. In a recently published meta-analysis compiling data from 47 studies on 1742 survivors of childhood ALL, the prevalence of overweight or obesity exceeded 40% in patients with <5 years of follow-up, ranged between 29 and 69% between 5 and 9 years and remained between 34 and 46% thereafter [27]. While the risk of obesity was highest in females and those treated with prophylactic CRT, more recent data from patients treated using contemporary chemotherapy-alone regimens have demonstrated that obesity remains a frequent and potentially major health issue in survivors of ALL [27]. Treatment with high-dose glucocorticoids appears to be the main risk factor for obesity and insulin resistance. The feasibility of early intervention to improve these outcomes represents an essential focus of future research.

Endocrine Late Effects in Survivors of Childhood Hodgkin's Lymphoma (HL)

HL is a highly curable malignancy in industrialized countries where it mostly develops in older adolescents (≥15 years) and young adults and has 5-year survival rates exceeding 90%. Patients with HL experience endocrine late effects because of treatment with alkylating agent chemotherapy and radiotherapy delivered to regions involved by lymphoma that are contiguous to endocrine glands in the neck, chest, abdomen or pelvis. Contemporary treatment protocols aim to reduce or eliminate radiotherapy given its significant association with late effects including cardiovascular diseases, second neoplasms and endocrine complications.

Disorders of the Thyroid

Survivors of pediatric HL with neck radiotherapy are at a significantly elevated risk of developing thyroid disorders including primary hypothyroidism, hyperthyroidism and thyroid neoplasia [28]. As frequently observed with other hormonal deficiencies associated with radiotherapy, the risk of hypothyroidism in pediatric HL survivors increases in a dose- and time-dependent fashion in relation to radiation and may exceed 50% at 20 years

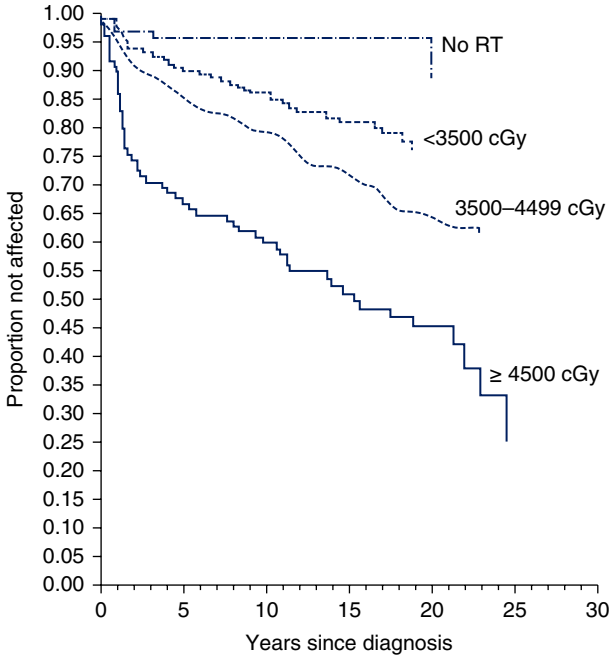

Figure 13.4 Probability of developing an underactive thyroid after diagnosis of Hodgkin's lymphoma [28]. *Source:* Reproduced with permission of Oxford University Press.

from diagnosis in patients treated with doses ≥45 Gy (Figure 13.4) [28]. Additional risk factors for hypothyroidism in survivors of pediatric HL include female sex and older age at diagnosis [28]. Hyperthyroidism has been less frequently reported than hypothyroidism in HL survivors, with a significantly higher risk than in the general population and highest among patients treated with doses of radiation over 35 Gy [28]. Thyroid neoplasia and cancer are a source of major concern in survivors of pediatric HL treated with radiotherapy. In a large cohort of long-term survivors of pediatric HL (median 15.5 years from diagnosis), thyroid cancer was diagnosed 18.4 times more frequently than expected [28]. The risk of thyroid cancer increases with radiation doses up to the 20–30 Gy range and then trends down at higher doses following an inverted U-shaped curve, likely because of significant damage to the gland at the highest doses. Alkylating agents may increase the risk of developing thyroid cancer in patients exposed to neck radiotherapy. Additional risk factors may include treatment for cancer before the age of 10 years.

Disorders of the Gonads

Males

Most males with HL maintain normal Leydig cell function and most of those with abnormalities have subclinical forms with raised LH and normal testosterone levels. Most survivors treated with alkylating agent

chemotherapy (Table 13.3) or with radiation fields potentially affecting the gonads are diagnosed with oligospermia or azoospermia [29]. Indirect evidence of germ cell failure was reported in nearly all patients treated with infra-diaphragmatic radiotherapy, while up to 70% of pediatric HL survivors treated with alkylating agent chemotherapy alone had evidence of azoospermia or oligospermia. The testicular toxicity of alkylating agents does not seem to correlate to age or pubertal stage at the diagnosis of HL [29]. Contemporary pediatric HL protocols seek to reduce the intensity of alkylating agent chemotherapy and radiation to decrease potential risk for gonadal toxicity; male patients should nevertheless be offered fertility preservation via sperm banking whenever feasible.

Females

Female HL survivors may develop premature ovarian insufficiency because of exposure to alkylating agents (Table 13.3) and/or pelvic radiotherapy. Older age at treatment represents an additional risk factor (Figures 13.2 and 13.3) [11], while oophoropexy may be protective in the subset of patients treated with pelvic irradiation. High rates of spontaneous resumption of menses after the completion of therapy and reports of pregnancies in females treated without pelvic irradiation are encouraging, but long-term follow-up data pertaining to the reproductive outcomes of survivors treated according to contemporary protocols remain scarce [29].

Abnormal Linear Growth and Body Composition

Survivors of pediatric HL may incur losses in their growth potential owing to the detrimental effect of spinal irradiation on the vertebral growth plates but these losses are on average moderate (–0.36 SD) even among patients treated with radiotherapy. Survivors of pediatric HL do not have an increased risk of developing significant BMD deficits. Limited data are available regarding other body composition changes in survivors of pediatric HL with some reports of relatively increased body fat and decreased lean mass [29].

Endocrine Late Effects in Survivors of Childhood Malignant Extra-Cranial Solid Tumours

The most common childhood extra-cranial malignant solid tumours are neuroblastoma, Wilms' tumour, bone sarcomas (osteosarcoma and Ewing's sarcoma), soft tissue sarcoma (rhabdomyosarcoma and embryonal sarcoma) and retinoblastoma. The risk of endocrine dysfunction in CCS treated for such tumours varies greatly according to tumour location and treatment exposure.

Growth Failure and HPA Dysfunction

Survivors of high-risk neuroblastoma frequently experience loss in growth potential because of the severity of their illness and the intensity of its treatment, which may include TBI. The epiphyseal growth plates may incur direct damage due to the use of high-dose localized radiation in some patients. Accelerated growth plate closure has also been reported following treatment with 13-cis-retinoic acid for high-risk neuroblastoma. GHD may contribute to growth failure in a subset of patients treated for high-risk neuroblastoma, while musculoskeletal sequelae such as scoliosis may further affect linear growth during adolescence [30]. Patients treated with radiotherapy for extra-cranial tumours of the head such as rhabdomyosarcoma, nasopharyngeal carcinoma or retinoblastoma may develop radiation-induced GHD and less commonly other HPA deficits and should be monitored for these dysfunctions similarly to patients treated with CRT for CNS lesions.

Thyroid Disorders

Primary hypothyroidism is among the most common endocrine late effects experienced by survivors of malignant childhood extra-cranial solid tumours. The prevalence is ~10% [31]. (131) I-metaiodobenzylguanidine (^{131}I-MIBG), a commonly used treatment modality for neuroblastoma, is known to be associated with a substantial risk of thyroid dysfunction because of radioactive iodine-induced glandular damage. Primary hypothyroidism may occur despite prophylactic treatment with potassium iodide (KI) and patients treated with ^{131}I-MIBG should be carefully followed with measurements of plasma free T4 (FT4) and TSH every 6 months during childhood or more frequently, as clinically indicated [32]. The risk of primary hypothyroidism and autoimmune disease may be increased by exposure to external beam radiation, including TBI. Primary hypothyroidism has also been described in patients treated with radiotherapy for rhabdomyosarcoma, nasopharyngeal carcinoma, retinoblastoma and other extra-cranial head and neck solid tumours. Patients treated with ^{131}I-MIBG for neuroblastoma have an increased risk of developing thyroid nodules [32]. Cases of papillary thyroid carcinoma have been reported in patients treated with ^{131}I-MIBG, without additional exposure to external beam radiation and despite prophylactic treatment with KI, and following treatment with radiotherapy for childhood head and neck rhabdomyosarcoma as well as other solid tumours.

Primary Gonadal Failure

Primary gonadal failure is the most common endocrine complication following malignant childhood extra-cranial solid tumours, affecting 9–11% of patients [31].

As with other childhood cancers, gonadal damage may occur because of the exposure to gonadotoxic chemotherapy such as alkylating agents (Table 13.3) or radiotherapy including TBI (Figures 13.2 and 13.3). More recently, primary ovarian insufficiency was described following the use of ^{131}I-MIBG for neuroblastoma. Patients with solid tumours within or near the gonads may develop primary hypogonadism because of tumour infiltration, surgical resection or gonadotoxic chemotherapy or radiotherapy. At-risk patients should be offered fertility preservation whenever feasible.

Overweight, Obesity, Glucose Intolerance and Primary Adrenal Insufficiency

Obesity does not occur at higher rates in survivors of childhood malignant extra-cranial solid tumours when compared with the general population [31]. Patients may have an increased risk of glucose intolerance and other components of the metabolic syndrome because of exposures to TBI and/or abdominal radiotherapy [30, 31]. Primary adrenal insufficiency does not seem to occur as a late effect in survivors of Wilms' tumour or neuroblastoma who had only one of their adrenal glands removed [33].

Diagnosis and Management of Common Endocrine Late Effects

Disorders of the Hypothalamo–Pituitary Axis

Hypothalamo-pituitary dysfunction may be present at the time of diagnosis because of tumours located within or near the HPA region and/or the surgery to remove such tumours (Table 13.1). HPA dysfunction may also occur as a late effect of tumour treatment following the direct or scatter exposure of the hypothalamus/pituitary region to radiation [3, 4]. The risk of HPA dysfunction increases in a dose- and time-dependent fashion in relation to radiotherapy and at-risk patients should be offered lifelong monitoring and follow-up.

Growth Hormone Deficiency

GHD should be suspected in CCS experiencing a sex- and age-adjusted linear growth velocity < −2 standard deviations (SD) over one year or <-1.5 SD over 2 years regardless of whether they have short stature. The linear growth of CCS may be affected by a variety of other endocrine and non-endocrine conditions and these require special attention. Spinal radiotherapy can directly damage the vertebral growth plates causing a particular form of skeletal dysplasia where spinal growth is more severely affected than that of the legs [34]. This can be detected by measuring the sitting height and comparing it with the standing height and sub-ischial leg length.

One can never overemphasize the necessity of monitoring closely the pubertal status of CCS throughout childhood and adolescence. Patients with tumours developing near the hypothalamus and those treated with CRT may concurrently develop CPP. Patients with CPP may experience falsely reassuring and seemingly normal growth rates, owing to sex steroid secretion, despite concurrently having GHD. These patients may incur irreversible losses in their growth potential as a result of rapidly fusing their growth plates if they are not diagnosed and treated expeditiously for both conditions [5]. Conversely, adolescents with GHD and hypogonadism require adequate replacement for both deficits to achieve an optimal response to treatment with human recombinant GH (hGH) and experience a pubertal growth spurt. Obesity may also influence linear growth, skeletal maturation and GH secretion.

The laboratory diagnosis of GHD in CCS relies on GH dynamic testing as in other populations. However, the consensus guidelines of the GH Research Society stipulate that failing one test (instead of the two tests required in the general population) is sufficient for the diagnosis in individuals with a history of CNS lesions or CRT [35]. It is important to note that the combination of GHRH and arginine cannot be used in patients with a history of CRT because of the probable hypothalamic cause of GHD in those individuals. Plasma insulin-like growth factor 1 (IGF-1) and IGF binding protein 3 (IGFBP-3) concentrations are frequently used as surrogate markers for GH secretion but they are not reliable in individuals with a history of exposure to CRT and their use may result in underdiagnosing GHD in at-risk CCS [36].

The growth prospects of children and adolescents with GHD can be substantially improved by treatment with recombinant human GH (rhGH). Individuals treated with CSI or TBI may not fully recover their precancer treatment growth predictions but rhGH seems to limit further erosion of growth potential. The safety of rhGH in individuals with a history of cancer has been the subject of several studies given the *in vitro* proliferative and pro-mitogenic properties of GH and IGF-1. Treatment with rhGH is not associated with higher risks of primary tumour recurrence or mortality in CCS [37]. Conflicting data exist regarding the association between treatment with rhGH and a higher risk of developing secondary solid tumours, especially meningiomas in patients treated with CRT [37]. A recently published statement from the North American Pediatric Endocrine Society (PES) supports the initiation of rhGH in pediatric CCS with GHD after the completion of all cancer treatments if they have no signs of active neoplastic disease and after

informing patients and their caregivers that there may be an increase in the risk of subsequent neoplasms [38].

Although evidence-based data are lacking regarding how long patients need to be monitored before rhGH is started, PES practice guidelines suggest waiting one year after the completion of cancer treatments; no specific recommendations were available for patients with craniopharyngioma [38]. Potential benefits on body composition, plasma lipids, bone mass and quality of life have extended the use of rhGH to adults with hypopituitarism. There are no studies specifically assessing the benefits and long-term risks associated with rhGH use after the completion of linear growth and in adult CCS; this continues to be an active area of research.

Central Precocious Puberty

The diagnosis and management of CPP in CCS are the same as in the general pediatric population. Additional elements specific to CCS should be taken into account. Clinicians should not rely on the measurement of testicular volume for the diagnosis of puberty in boys treated with high-dose alkylating agents or direct testicular radiotherapy as these patients may have small testes resulting from treatment-induced germ cell injury without losing the ability to produce testosterone. Other symptoms such as pubarche, scrotal thinning and penile size may be useful and practitioners should not hesitate to measure morning testosterone and LH plasma concentrations for confirmation. Clinicians should be aware of the possible and frequent association between CPP and GHD as well as with other pituitary deficiencies and endocrine late effects [7].

Long-term follow-up data are increasingly available on CCS with a history of CPP. Patients with CPP as a result of a CNS tumour or CRT may not fully recover their growth potential despite early diagnosis, treatment and replacement of associated hormonal deficiencies. An average 0.9 SD loss in the final height, more likely to be attributable to tumour burden rather than to CPP, has been reported [7]. Patients with a history of CPP treated with CRT may paradoxically experience LH/FSHD as a radiation-induced late effect years later and require sex hormone replacement therapy [7]. Patients may also experience high rates of obesity and gonadal dysfunction (females), but these are also more likely to be related to tumour and treatment factors rather than CPP and pubertal suppression based on reassuring long-term follow-up data from individuals with idiopathic forms of CPP [7].

Hypogonadotropic Hypogonadism

Treatment of gonadal failure secondary to LH/FSHD relies on sex hormone replacement therapy adjusted to pubertal stage. The management of reproductive dysfunction secondary to LH/FSHD requires germ cell (males) or follicular growth (females) stimulation by specialized reproductive medicine care providers [39, 40].

Central Hypothyroidism

Symptoms of TSHD are similar to those of primary hypothyroidism; TSHD should be suspected when plasma FT4 concentrations below normal coincide with low or inappropriately normal TSH values. Treatment of TSHD relies on the use of levothyroxine and aims at maintaining plasma FT4 values within the middle to upper parts of the normal range. Measuring plasma TSH is inappropriate to adjust treatment with levothyroxine in patients with a known diagnosis of TSHD. In the context of multiple pituitary hormone deficiencies, clinicians should remember to initiate treatment for ACTHD before that of TSHD given that thyroid replacement increases the clearance of endogenous cortisol and may induce an adrenal crisis in patients with undiagnosed adrenal insufficiency.

Central Adrenal Insufficiency

Patients with ACTHD may experience the typical symptoms of adrenal insufficiency and are at risk of seizures, hypoglycaemia and shock if they are not treated promptly with higher parenteral stress doses of glucocorticoids during episodes of severe illness. The diagnosis may be suspected by lower than normal 8 AM plasma cortisol concentrations and by dynamic testing, the low-dose ACTH stimulation test being the most commonly used modality. In patients with multiple hormone deficiencies (e.g. following CRT and CSI), clinicians should remember to initiate treatment for ACTHD before that of primary hypothyroidism or TSHD. The treatment of ACTHD includes using maintenance doses of hydrocortisone and teaching patients and their families how to escalate treatment in situations of illness. Patients with ACTHD should carry some form of documentation (via cards, necklaces, bracelets, etc.) indicating that they have adrenal insufficiency at all times.

Disorders of the Thyroid

Primary Hypothyroidism

The diagnosis and management of primary hypothyroidism in survivors of childhood CNS tumours are similar to those practised in the general population with two specific recommendations (Table 13.2). Levothyroxine therapy for subclinical, compensated, primary hypothyroidism (manifesting through elevated TSH and normal FT4 concentrations) in patients with a history of exposure to radiation may be justified given the potential association between TSH elevation and thyroid neoplasia [41]. It is also important to remember that patients

with a history of CRT or CSI may concomitantly develop TSHD and that in such mixed forms of hypothyroidism, suppressed plasma TSH concentrations are more likely to indicate hypothalamo-pituitary dysfunction rather than overdosing with levothyroxine. In such patients who are at risk of developing multiple hormone deficiencies, medical providers should remember to assess the hypothalamo–pituitary–adrenal axis and initiate treatment for ACTHD if needed before that of primary hypothyroidism. At-risk patients should be offered lifelong monitoring of thyroid function as primary hypothyroidism may occur decades after the completion of cancer therapy.

Thyroid Neoplasia

Screening guidelines continue to be a topic of controversy in CCS at risk of thyroid cancer [42, 43]. The COG presently recommends screening at-risk patients with yearly clinical neck palpation by an experienced provider (www.survivorshipguidelines.org). The use of thyroid ultrasound for screening purposes remains controversial given high rates of false positives and uncertain benefits in terms of improving long-term health outcomes [42]. Some have argued in favour of ultrasound screening of very high-risk patients, citing the potential benefit of decreasing disease burden and treatment-related morbidity with early diagnosis [43]. Other expert panels have explicitly refrained from endorsing or discouraging the use of ultrasound for screening patients at risk of thyroid neoplasia [44].

Thyroid cancer following treatment for pediatric malignancies should be investigated and treated as primary thyroid cancers encountered in the general population [44]. Thyroid cancer does not seem to present or behave more aggressively as a second malignancy than when it is diagnosed as a primary cancer [42]. Total thyroidectomy is the preferred surgical procedure for the treatment of thyroid cancer in patients with a history of neck radiation given the risk of neoplasia on an irradiated remnant [44].

Disorders of the Gonads

Males

The diagnosis and management of Leydig cell failure in CCS are similar to those practised in the general population (Table 13.2). In contrast to testosterone production, spermatogenesis may be affected by chemotherapy and by CSI regimens [9]. Diagnosing abnormal spermatogenesis requires semen analysis given the limited reliability of plasma markers such as FSH and inhibin B. Patients at risk of germ cell failure should be offered the option of sperm banking prior to treatment with gonadotoxic agents [10].

Females

The diagnosis and management of primary ovarian insufficiency in CSS are similar to those practised in the general population. Females with primary ovarian insufficiency should be offered sex hormone replacement therapy as they would otherwise be at risk of bone mineral density deficit, increased cardiovascular morbidity and impaired quality of life [40]. Fertility preservation by mature oocyte cryopreservation is no longer regarded as experimental and may represent a viable option for young pubertal CCS at risk of primary ovarian insufficiency [10]. Ovarian tissue cryopreservation carries the risk of tumour cell contamination and cancer cell reseeding at reimplantation; this procedure remains experimental and is not recommended for use in female CCS treated for haematological malignancies [10]. Even when they are able to become pregnant, female CCS exposed to pelvic radiotherapy or TBI have an increased risk of premature delivery, miscarriage or having low birth weight newborns, possibly because of the effects of irradiation on the uterus and its vascular supply [20]. Research regarding birth outcomes has demonstrated that the offspring of female CCS do not experience higher than expected rates of congenital malformations or chromosomal abnormalities [45].

Bone Mineral Density Deficit

The COG screening guidelines support obtaining an assessment of BMD via DXA or qCT in at-risk patients upon entry into long-term follow-up and subsequently as clinically indicated (www.survivorshipguidelines.org) (Table 13.2). The management of low BMD in CCS includes general counselling for healthy lifestyle habits (adequate dietary intake of calcium, regular physical activity, cessation of smoking/reduced alcohol consumption) and making sure that hormonal deficiencies, including vitamin D insufficiency, are adequately screened for and replaced.

Overweight, Obesity and Glucose Intolerance

Overweight and obesity should be detected using auxological markers (height, weight and BMI) as in the general pediatric population (Table 13.2). It is important to note, however, that abnormal body fat distribution rather than overall increases in BMI has been associated with increased cardiovascular risk in a subset of CCS, especially those treated with HSCT. Regardless of whether BMI falls in the overweight/obese range or not, patients with a history of HSCT treated with TBI need to be regularly screened (every 2 years as per the COG in the absence of additional risk

factors) using fasting laboratory measurements of plasma glucose, lipids and haemoglobin A1c. Despite the atypical presentation of these patients, there are at present no specific guidelines for the management of their metabolic derangements. Further research is needed to inform the best clinical approach to insulin insensitivity and diabetes mellitus in HSCT survivors given the high rates of premature death from cardiovascular causes in this population.

Increased parasympathetic tone and ensuing hyperinsulinemia were described as potential mechanisms for hypothalamic or central obesity in CNS tumour survivors, and treatment with octreotide has been suggested as a possible therapeutic approach [46]. Stimulant medications, e.g. dextroamphetamine, have also been shown to stabilize BMI in small cohorts with hypothalamic obesity [47]. These pharmacological treatments have not been widely adopted given the limited data supporting their long-term safety and efficacy.

Preparing for the Transition to the Adult Care Setting

Planned transition of patients from the pediatric to the adult care setting has gained wide recognition as a goal for enhancing the quality of care. Numerous studies of chronic diseases of childhood have highlighted improved outcomes brought about by transition planning and many initiatives such as the federally supported National Healthcare Transition Center (NHTC) in the USA have been strongly advocating a systematic approach (www.gottransition.org). This is particularly relevant to the CCS population given the chronic nature of many of the endocrine complications diagnosed during childhood and the need for lifelong follow-up and monitoring of CCS who are at risk of developing them as late effects during adulthood.

Available data regarding the present status of the transition of endocrine care of CCS are concerning for a high prevalence of undiagnosed and/or untreated conditions after patients leave their treating pediatric institutions and potential repercussions on physical fitness and cardiovascular health [3]. The transition process should be initiated by the pediatric care providers at least one year ahead of the anticipated transfer date in order to allocate enough time for completing patient education, setting up the initial appointment with the adult care practice and transferring necessary information and documentation. The NHTC encourages pediatric care providers to verify the completion of the transition process by contacting directly the practice to where their patients were referred within 3 months of the scheduled date of transfer.

Endocrine Complications and Novel Cancer Therapies

Chemotherapy Agents

In contrast to more traditional cytotoxic chemotherapies, novel treatment strategies for cancer include targeted agents designed to inhibit tumour growth by interfering with specific molecular pathways. Tyrosine kinase inhibitors (TKIs) and immunomodulators, such as interferon and immune checkpoint inhibitors, are increasingly used in pediatric cancer treatment protocols. For instance, imatinib mesylate, a TKI targeting the Bcr-Abl mutation, has become a front-line drug in the management of chronic myeloid leukaemia (CML) in children. Despite intending these medications to target pathways believed to be critical for tumour growth, endocrine and skeletal growth disruptions associated with their use have already been reported. The prolonged use of some of these medications as maintenance therapy creates the need to screen for and manage endocrine disorders known to be associated with their usage as potential late effects.

Growth failure has been reported in children during treatment for CML with imatinib, with speculation regarding the respective contributions of GHD, resistance to GH or IGF-1 and direct skeletal toxicity to this adverse outcome [48]. Even in children with proven GHD while on imatinib, treatment with GH is presently contraindicated in patients with active malignancies [38]. Additional research may further elucidate the full impact of TKIs on linear growth and help determine strategies such as changes in the drug delivery regimen that may avoid adverse effects on endocrine function. Other HPA deficits have anecdotally been reported in patients on imatinib, including ACTHD; patients should be monitored for these as clinically indicated [48].

Immune checkpoint inhibitors are another class of targeted agents that may potentially cause HPA deficits [49]. These agents may enhance autoimmunity against endocrine glands as they act by disrupting the immune system's tolerance of tumour cells [49]. For instance, anti-CTLA4 monoclonal antibodies (such as ipilimumab), which are increasingly used for the treatment of unresectable melanoma, have been reported to induce hypophysitis in a substantial number of patients, depending on the dose and regimen used. Patients may present with ACTHD, LH/FSHD, TSHD or GHD within a few weeks of starting ipilimumab with deficiencies persisting in more than 50% of cases after discontinuation of treatment [49]. Clinicians need to be aware of the risk of adrenal insufficiency in particular and patients should be screened and treated as clinically indicated [49]. Other targeted agents such as retinoic acid may interfere directly with skeletal growth; continued research is

crucial in order to understand the benefits and harms of using such therapies in children [50].

Primary thyroid disease has been extensively described in patients treated with TKIs with hypothesized mechanisms including thyroiditis, changes in iodine uptake and capillary vascularization of the gland [48]. Primary hypothyroidism has been associated with the use of several TKIs and is particularly common in patients treated with imatinib, sunitinib and sorafenib [48]. Patients treated with immunomodulators including pegylated interferon and anti-CTLA4 monoclonal antibodies such as ipilimumab and bevacizumab have also an increased risk of autoimmune thyroiditis and primary hypothyroidism [49]. Patients on these treatments should be screened for autoimmune thyroiditis at protocol entry by measuring plasma thyroid autoantibodies and FT4 and TSH concentrations; thyroid function tests should be regularly repeated thereafter. Screening at day 1 of each chemotherapy cycle is suggested [49] or even more frequently if patients have positive autoantibodies. Treatment with levothyroxine should be initiated in patients with symptomatic or decompensated hypothyroidism. Unlike radiation-induced primary hypothyroidism, the treatment of patients with subclinical hypothyroidism (isolated TSH elevation) due to exposure to targeted agents is controversial [48].

Other hormonal dysfunctions such as hypo- and hyperglycaemia, decreased BMD, primary adrenal insufficiency, gynaecomastia and primary gonadal failure have been reported in patients treated with TKIs [48]. The long-term effects on the endocrine system of TKIs, as well as that of other targeted therapies, have not been fully elucidated given that these medications are relatively new. Such data will become increasingly available with continued monitoring and the long-term and prospective follow-up of patients [48, 49].

Proton Beam Radiotherapy

Technical improvements are continuously being sought to reduce the exposure of normal organs, including endocrine glands, to scatter radiation. In this regard, proton beam radiotherapy instead of the more widely available conformal photon-based techniques seems promising. Long-term data comparing endocrine outcomes in children treated with these 2 modalities are limited. Patients treated with proton beam radiotherapy for brain tumours located outside of the hypothalamo–pituitary area seem to have a lower risk of developing endocrine dysfunction compared with those treated with photon-based radiation in the short to medium term, although longer-term follow-up data are necessary given the time- and dose-dependent nature of these late effects [51]. Additional data are needed regarding the outcomes of patients with craniopharyngioma and other sellar or suprasellar tumours with the understanding that direct damage related to the tumour and/or surgery would complicate their interpretation. Benefits of using proton beam radiotherapy in limiting radiation-related injury to the vertebral growth plates, the thyroid and the gonads in patients treated with CSI or localized radiotherapy for various non-CNS malignancies and solid tumours deserve further study.

Summary

Endocrine late effects are among the most common long-term complications experienced by survivors of childhood cancers. Prospective and systematic risk-based screening should allow the early diagnosis of endocrine conditions and expeditious treatment to avoid additional health morbidities among these vulnerable patients. The importance of lifelong follow-up of patients at risk of endocrine late effects cannot be overemphasized since even novel treatment strategies using targeted chemotherapy agents or proton beam radiotherapy do not eliminate the risk of hormonal dysfunction. Prospective, multicentre and international collaborations are encouraged in order to keep abreast of new knowledge in this ever-evolving field.

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Weblinks

Children's Oncology Group Long-term Follow-up Guidelines: www.survivorshipguidelines.org

Children's Cancer and Leukaemia Group – http://www.cclg.org.uk/dynamic_files/LTFU-full.pdf

Scottish Intercollegiate Guidelines Network – http://sign.ac.uk/pdf/sign132.pdf

The National Healthcare Transition Center – http://www.gottransition.org

14

Disorders of Water Balance

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KEY LEARNING POINTS

- In diabetes insipidus, large volumes of dilute urine (polyuria) are excreted due to vasopressin (AVP) deficiency (central diabetes insipidus [CDI]), vasopressin resistance (nephrogenic diabetes insipidus [NDI]) or excessive water intake (primary polydipsia).
- The maintenance of water balance in healthy humans is achieved by three determinants: thirst, vasopressin and kidney function. Renin–angiotensin–aldosterone and natriuretic peptides also regulate water and electrolyte balance.
- Vasopressin and its protein carrier NPII are released from the posterior pituitary by calcium-dependent exocytosis when the axon is depolarized by osmoreceptor or baroreceptor stimuli.
- Water gets through the cell membrane by aquaporin, a water channel. AVP activates the insertion of aquaporin 2 vesicles into the cell membrane of the collecting duct.
- CDI may be due to the destruction of neurons originating in the supraoptic and paraventricular nuclei because of intracranial germ cell tumours, Langerhans cell histiocytosis, inflammatory/autoimmune conditions, vascular diseases, trauma resulting from surgery or an accident, metastases and midline cerebral and cranial malformations and other rare conditions. In other cases, genetic defects in AVP synthesis or other genetic diseases are the underlying cause.
- Nephrogenic diabetes insipidus is secondary to AVP receptor-2 mutations or abnormalities of the gene encoding aquaporin 2 water channels.
- History, patient's age at disease onset and clinical examination may provide important clues to possible underlying diagnoses.
- Polyuria must be evaluated by means of a water deprivation test, with measurement of plasma and urine osmolality, and DDAVP challenge should be performed if diabetes insipidus has been diagnosed.
- Magnetic resonance imaging with current high field systems represents the modality of choice for evaluating the hypothalamic–pituitary axis in CDI. Pituitary stalk size evolution should be monitored. Additional important diagnostic information can be obtained by clinical, radiological, biochemical and endocrine follow-up studies.
- Patients with idiopathic CDI and a thickened pituitary stalk are likely to develop anterior pituitary hormone deficits.
- The drug of choice for the treatment of CDI is oral, intranasal or parenteral desmopressin (dDAVP). Dilutional hyponatraemia is a potential hazard if desmopressin is administered in excess over a prolonged time period.
- Volume contraction and thiazide diuretics, amiloride and indomethacin are indirect forms of treatment for NDI.
- The syndrome of inappropriate antidiuretic hormone secretion (SIADH) and cerebral salt-wasting syndrome (CSWS) are causes of hyponatraemia. Clinical evaluation, assessment of extracellular fluid space volume status, measurement of urinary electrolytes and responses to infusion of saline solutions can distinguish these conditions; therapy (water restriction/oral urea/vaptans or saline infusion) must be carefully chosen after determining the cause of hyponatraemia.

The maintenance of the tonicity of extracellular fluids within 1–2% of normal (287 ± 7 mosm/kg) is crucial for cell function, and so homeostasis by the regulation of water intake and excretion is critical to all mammals. This requires the normal function of the hypothalamus and its surrounding brain tissue, the posterior pituitary secreting arginine vasopressin peptide (AVP) and the kidney. AVP is a nonapeptide produced by the magnocellular neurons of the supraoptic and paraventricular nuclei (PVN) of the hypothalamus and released from the posterior pituitary with its associated protein (neurophysin, NPII) by calcium exocytosis. Vasopressin is the principal hormonal regulator of urine volume and concentration and the controller of water homeostasis under normal conditions. Disorders of vasopressin secretion and of its action in the kidney are associated with disrupted water metabolism leading to diabetes insipidus.

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Regulation of Water Balance

Anatomy of the Hypothalamic–Posterior Pituitary Axis

The posterior pituitary consists of magnocellular neurons producing vasopressin and/or oxytocin. The cell bodies are located in the paraventricular and the supraoptic nuclei (SON) in the hypothalamus and axons project to the neurohypophysis where the hormones are stored and secreted into the bloodstream. These axons store quantities of vasopressin large enough to sustain basal release for 30–50 days or to allow maximal antidiuresis for 5–10 days [1].

While the blood supply to the anterior pituitary (AP) is through the hypothalamo-pituitary portal system from the suprahypophyseal arteries, the vascularization of the posterior pituitary is directly from the inferior hypophyseal arteries, which are branches of the posterior communicating and internal carotid arteries. Drainage is into the cavernous sinus and internal jugular vein. The adult neurohypophysis weighs on average 120 mg, with the weight increasing slightly with age.

During embryogenesis, neuroepithelial cells of the lining of the third ventricle migrate to the walls of the third ventricle where they mature into PVN. Some cells continue to migrate laterally to and above the optic chiasm to form the SON. Their unmyelinated axons traverse the basal hypothalamus and form the neural stalk; their terminations form the posterior pituitary. The SON ultimately contain *only* oxytocin and vasopressin-containing neurons, whereas the PVN have cells that contain corticotropin-releasing hormone (CRH), TSH-releasing hormone (TRH), somatostatin and neurotransmitters destined to control the autonomic nervous system [2].

The early differentiation of these cell lineages has become clearer through the elucidation of the role of transcription factors in hypothalamic development. Sim1, Arnt2, Otp and Brn2 appear to be involved in the cascade of transcription factors implicated in the development of the neuroendocrine hypothalamus leading to the completion of posterior pituitary development by the end of the first trimester, when vasopressin and oxytocin can be detected in neurohypophyseal tissue [3]. Loss of ARNT2 function has a profound impact on normal central nervous system development, especially the hypothalamus and pituitary gland [4]. The combination of multiple pituitary hormone deficiencies observed in six children born within a consanguineous family of Saudi Arabian origin who presented in the first month of life with hypernatraemia secondary to central diabetes insipidus (CDI) is consistent with a key role for *ARNT2* in the development of specific neurosecretory neurons in the human hypothalamus.

The posterior pituitary gland is formed by the evagination of neural tissue from the floor of the third ventricle. It consists of the distal axons of the hypothalamic magnocellular neurons that form the neurohypophysis. After its downward migration it is encapsulated with the ascending ectodermal cells of Rathke's pouch, which form the AP. In a recent study a *Hes1*-null pituitary gland was revealed to be reduced in size but was otherwise morphologically normal compared with the control. In *Hes1–Hes5* double-mutant mice, the evagination of the infundibulum was affected and the neurohypophysis was lost compared with both the wild-type and *Hes1*-null mice, suggesting that both *Hes* genes are essential for the formation of the neurohypophysis [5].

A number of transcription factors have been implicated in the development of the hypothalamo-neurohypophyseal system (HNS) and null mutations for these factors caused severe defects in proliferation, migration and survival during early embryogenesis [6, 7]. Large numbers of genes have been identified in rat HNS neuronal tissues after dehydration. The pattern of HNS transcripts with marked differences in gene expression indicates that these genes are candidate regulators and effectors of HNS activity and remodelling [8].

Neurons, Periphery and Regulation of Thirst

Thirst is the instinct to drink water. Body fluid homeostasis regulates the internal salt and water balance and, as this balance shifts, the brain senses the changes and triggers specific goal-oriented intake behaviour [9]. Recent advances in understanding the molecular, cellular and network mechanisms that mediate the central control of osmotic homeostasis in mammals [10] have shown that neurons in several circumventricular organs (CVO) of the hypothalamus are activated by thirst-inducing conditions: dehydrated animals are strongly motivated to consume water [11]. Intracranial injection of angiotensin, a vasoactive hormone that stimulates drinking, has been shown to activate CVO neurons in several species, and electrical stimulation of CVO nuclei increased fluid consumption in rodents.

Two distinct neural populations in the subfornical organ (SFO) that trigger or suppress thirst have been identified. When one population of SFO neurons are activated, they evoke intense drinking, even in fully water-satiated animals. By contrast, activation of another population of SFO neurons drastically suppresses drinking, even in water-craving thirsty animals. These results reveal an innate brain circuit that can turn an animal's desire to drink water on and off and the SFO probably functions as a centre for thirst control in mammals [12].

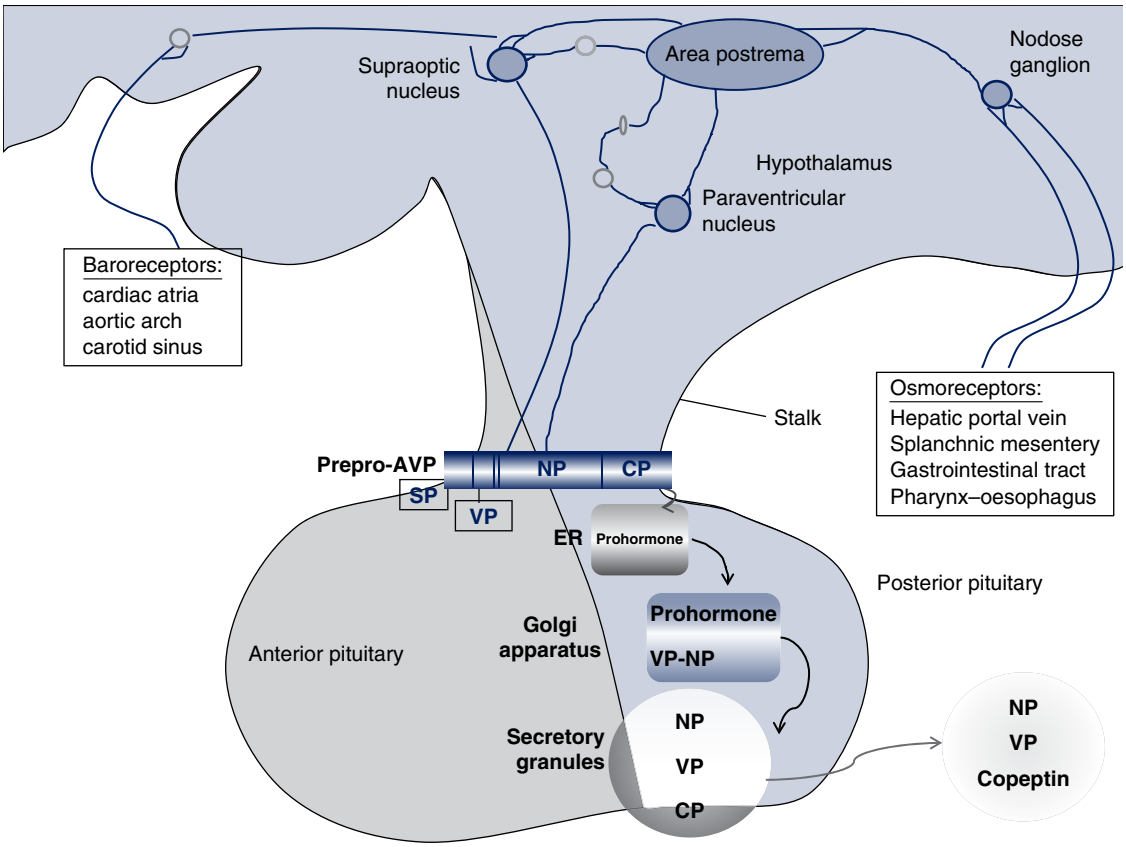

Figure 14.1 Osmoreceptor and baroreceptor circuits and AVP synthesis and secretion in mammals. SP, signal peptide; VP, vasopressin; NP, neurophysin; CP, copeptin; ER, endoplasmic reticulum.

In both animals and humans, there are peripheral osmoreceptors in the upper regions of the alimentary tract and in the blood vessels that collect solutes absorbed from the intestine [10]. These receptors are located in the oropharyngeal cavity, the gastrointestinal tract, the splanchnic mesentery, the hepatic portal vein and the liver; the osmoregulatory circuits in the mammalian brain and the periphery are shown in Figure 14.1. The information collected by the osmoreceptors reaches the central nervous system through fibres that ascend in the vagus nerve [10].

Vasopressin Biosynthesis

The AVP-neurophysin II gene (*AVP-NPII*) is located distally on the short arm of chromosome 20 (20p13). It covers 2.5 kb and comprises three exons. Exon 1 encodes the signal peptide of 19 amino acid residues, the nonapeptide AVP and the N-terminal region of NPII (9 amino acid residues); exon 2 encodes the central highly conserved region of the NPII peptide (67 amino acid residues); exon 3 encodes the C-terminal region of NPII (17 amino acid residues) and a 39 amino acid glycopeptide known as copeptin [13].

The *AVP-NPII* gene product, the AVP preprohormone, is co-translationally targeted to the endoplasmic reticulum (ER) where the signal is cleaved by signal peptidase and the copeptide is core glycosylated. Vasopressin and NPII associate after cleavage and then form a tetramer that increases the binding affinity of vasopressin for NPII. After formation of seven disulphide bonds within NPII and one within AVP, and after glycosylation of the copeptide, the pro-precursor is packaged into neurosecretory granules and then cleaved into the product peptides during axonal transport to the posterior pituitary [13].

Neurophysin stabilizes the hormone during its transport and storage but copeptin is also important for the correct structural formation of the AVP precursor as a prerequisite for its efficient proteolytic maturation [14]; indeed, copeptin has emerged as a promising marker for the diagnosis of AVP-dependent fluid disorders [15].

Vasopressin and NPII are released from the posterior pituitary by calcium-dependent exocytosis when the axon is depolarized by osmo- or baroreceptor stimuli (Figure 14.1). Once in the circulation, vasopressin has a half-life of only 5–10 minutes due to its degradation by a vasopressinase. During pregnancy, AVP secretion and

thirst occur at a lower serum osmolality. In addition, placental vasopressinase/oxytocinase, a leucine aminopeptidase inactivating AVP, oxytocin and other small peptides, is produced by 7 weeks' gestation by trophoblasts and reaches maximal levels in the third trimester. Consequently, clearance reaches a plateau at a fourfold higher rate by 22–24 weeks and remains at this level until delivery, thereby prompting a compensatory rise in AVP synthesis and secretion. Serum vasopressinase activity correlates with placental weight and is higher in pregnancies with more than one fetus. Increased vasopressinase activity can sometimes result in transient gestational DI that remits after delivery [16].

Physiology of Water Homeostasis

The maintenance of water balance is achieved by thirst, vasopressin and kidney function. In addition, apelin, a bioactive peptide, has been isolated from bovine stomach extracts (like ghrelin, another stomach–hypothalamus association). It is expressed in the supraoptic and PVN and exerts its action on specific receptors located on vasopressinergic neurons. Apelin is a potent diuretic neuropeptide counteracting vasopressin actions through inhibition of AVP neuron activity and AVP release. The coexistence of apelin and AVP in magnocellular neurons and their opposite biological effects and regulation are likely to play a key role in fluid homeostasis [17].

As early as the middle of the nineteenth century, the question was asked: how does water get through the lipid bilayer of the cell membrane? The answer used to be '*…membranes have pores*', but in 1988 a membrane protein called aquaporin was described as the long-sought-after water channel [18]. This discovery enabled a series of biochemical, physiological and genetic studies of water channels in bacteria, plants and mammals that explain how a water molecule passes through the cell membrane and why only water and no other small molecules or ions can pass through the membrane. In 2000, Agre reported the first high-resolution images of the three-dimensional structure of aquaporin enabling a detailed map of water channel functions to be constructed.

Vasopressin acts on its major target organ, the kidney, where it increases urine osmolality (Figure 14.2). The hormone binds to the V2 receptors in the basolateral membrane of the collecting tubule and activates the

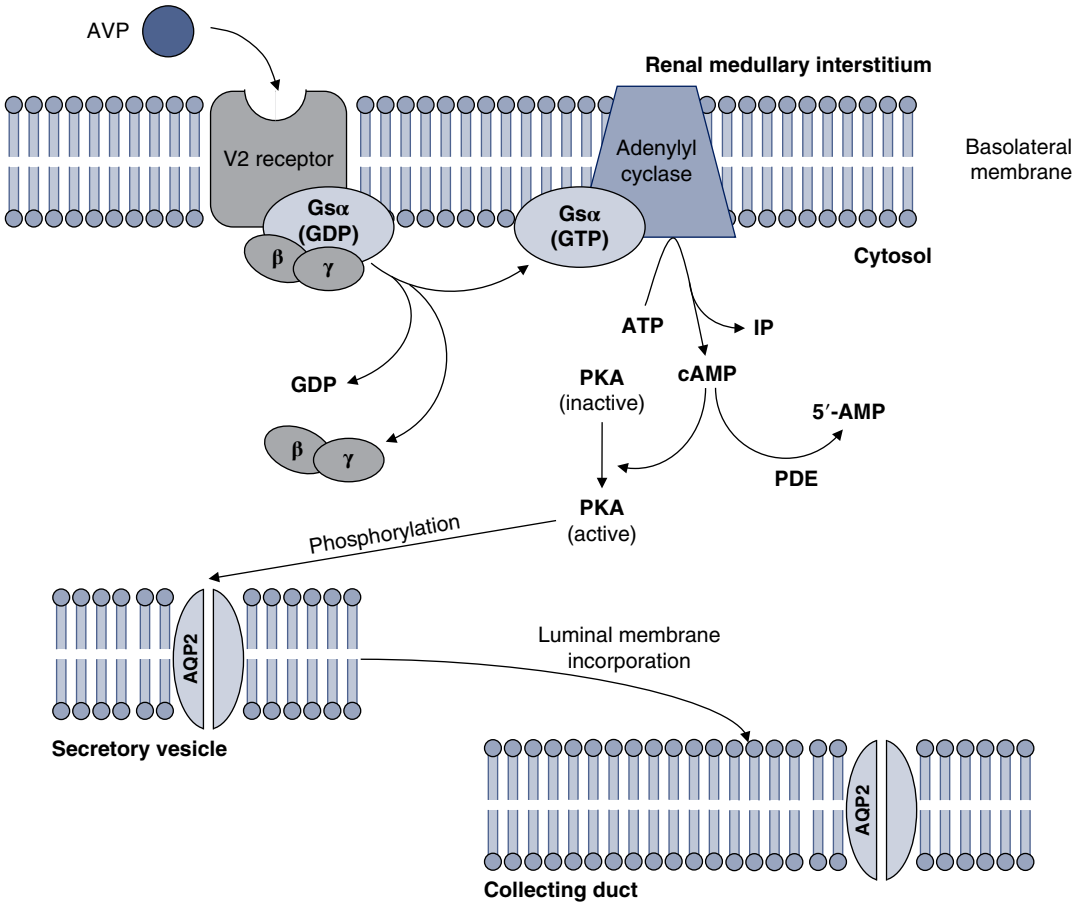

Figure 14.2 AVP action on renal collecting duct cells.

G_s–adenylcyclase system, increasing intracellular levels of cyclic 3′,5′-adenosine monophosphate (cAMP). The latter activates protein kinase A, which in turn phosphorylates preformed acquaporin-2 (AQP2) water channels localized in intracellular vesicles. Phosphorylation promotes trafficking to the apical membrane, followed by exocytic insertion of AQP2 vesicles into the cell membrane. The insertion of AQP2 renders the collecting duct water permeable by allowing free movement of water from the lumen of the nephron into the cells of the collecting duct along an osmotic gradient, thus concentrating the urine. The synthesis of AQP2 channels and their movement is regulated by AVP stimulation, while acquaporin-3 and acquaporin-4, responsible for the subsequent passage of water from within the cell into the renal interstitium, are constitutively present in the basolateral membrane [19].

Regulation of Vasopressin Secretion

Osmotic Regulation

The maintenance of water balance begins with sensing plasma osmolality predominantly represented by the plasma sodium concentration. The sensing mechanism is controlled by specialized neural osmoreceptors in the anterolateral hypothalamus responsible for AVP production and secretion. The osmoreceptors are quiescent below a plasma threshold osmolality of about 280 mosm/kg H_2O. When plasma osmolality rises above this threshold value, osmoreceptor cells are progressively stimulated to release AVP.

Small changes in plasma osmolality regulate AVP release from the posterior pituitary. When water is lost and plasma osmolality increases by as little as 1%, an increased secretion of AVP stimulates water retention by the kidneys. There is a sensitive, linear relationship between increased osmolality and increased AVP secretion; there is a similar linear relationship between increased plasma AVP and increased urine osmolality. Osmolality is tightly regulated around each individual's normal value that falls between 280 and 295 mOsm/kg H_2O in the general population [20]. Maximal antidiuresis is attained with plasma AVP concentrations around 2–5 pmol/L.

When individuals lose large amounts of body water, plasma osmolality may rise above 300 mOsm/kg H_2O but increased AVP secretion of more than 5 pmol/L cannot further concentrate urine (1000–1200 mOsm/kg H_2O). Urine volume does not change markedly over wide variations of urine osmolality until urine osmolality approaches maximum dilution and plasma AVP is completely suppressed. There is then a remarkable exponential increase in urine volume to ~18 L/day in adults. The glomerular filtrate is largely reabsorbed in the descending loops of Henle in the kidneys and only ~18 L of dilute fluid enters the collecting duct.

Non-Osmotic Regulation

Vasopressin is also released in response to non-osmotic stimuli, including hypovolaemia, hypotension, stress, nausea and drugs (Table 14.1) [21]. AVP release is under

Table 14.1 Regulation of vasopressin secretion.

Mechanism	Receptor	Causes
Osmotic	• Hypothalamic osmoreceptors	• Plasma osmolality • Hyperglycaemia • Hypertonic/hypotonic solution infusion • Water balance change
Hemodynamic (renin–angiotensin–aldosterone system, natriuretic peptide system)	• High pressure arterial baroreceptor (carotid sinus, aortic arch) • Low-pressure volume receptors (atria and pulmonary venous system)	• Blood volume/hypovolaemia/haemorrhage • Blood pressure • Vasovagal reaction • Congestive heart failure • Cirrhosis • Nephrosis • Pregnancy
Emetic	• Area postrema of the medulla "chemoreceptor trigger zone"	• Nausea
Other		• Drugs (morphine, vincristine, cyclophosphamide, nicotine, carbamazepine, glucocorticoids, ethanol, etc.) • Temperature • Stress

the control of effective circulating volume. The baroreceptor systems responsible for controlling the sympathetic nervous system also control AVP secretion. These neural pathways originate from both the low-pressure sensors mainly in the cardiac atria and the high-pressure sensors mainly in the aortic arch and carotid sinus. Afferent stimuli are carried by the vagal and glossopharyngeal nerves, with primary synapses in the nucleus tractus solitarius. Secondary projections then relay signals regarding circulatory fullness to the neurons in the hypothalamic nuclei that control AVP synthesis and secretion.

Renin–Angiotensin–Aldosterone System

This system is central to the control of salt and water balance and arterial blood pressure [22]. The activity of the system is controlled by renin, which is released from juxtaglomerular epithelioid cells into the circulation. Renin release is regulated by a complex interplay of several locally acting hormones or mechanisms and longer feedback loops, one of which involves salt intake. Acute salt loads or chronic high salt intake suppresses plasma renin activity, whereas reductions in salt intake stimulate it. Because the activation of the system conserves the salt content of the body, a classic feedback loop between salt intake/body salt content and renin is established.

Despite its important role for body fluid homeostasis, the precise signalling pathways connecting salt intake with the synthesis and release of renin are incompletely understood. Four controllers of the salt-dependent regulation of the renin–angiotensin–aldosterone system have been suggested:

1) The macula densa mechanism, which adjusts renin release in response to changes in the renal tubular salt concentration.
2) Salt-dependent changes in arterial blood pressure.
3) Circulating salt-dependent hormones, particularly the atrial natriuretic peptide (ANP).
4) Renal sympathetic nervous activity, which is regulated by extracellular volume and arterial blood pressure [22].

The Natriuretic Peptide System

The natriuretic peptides (NP) are a group of proteins synthesized and secreted by the mammalian heart [23, 24]. All are synthesized from prohormones and have 17-amino acid cyclic structures containing two cysteine residues linked by internal disulphide bonds. They are characterized by a wide range of actions mainly through their membrane receptors. The NP regulate water and electrolyte balance and blood pressure through their diuretic and natriuretic effects and by causing vascular smooth muscle relaxation. They also affect the endocrine and nervous systems. The results of the neurohormonal regulation of blood circulation are based mainly on antagonism with the renin–angiotensin–aldosterone system.

The balance between vasoconstrictor/sodium-retaining and vasodilator/natriuretic systems is essential for maintaining body fluid and electrolyte homeostasis. NP, such as ANP, brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP), belong to the vasodilator/natriuretic system and contribute to salt and water balance. In particular, ANP is produced by the conversion of pro-ANP into ANP, which is achieved by a proteolytic cleavage. In the kidney, ANP binds to the natriuretic peptide receptor-A and enhances its guanylyl cyclase activity, thereby increasing intracellular cyclic guanosine monophosphate production to promote natriuretic and renoprotective responses. In the glomerulus, ANP increases glomerular permeability and filtration rate and antagonizes the deleterious effects of the renin–angiotensin–aldosterone system activation [23, 24].

Diabetes Insipidus (DI)

Definition of DI

In DI large volumes of dilute urine (polyuria) are excreted due to vasopressin (AVP) deficiency (CDI), vasopressin resistance (nephrogenic diabetes insipidus [NDI]) or excessive water intake (primary polydipsia). Polyuria is characterized by urine volume in excess of 2 L/m^2/24 h or ~150 mL/kg/24 h at birth, 100–110 mL/kg/24 h up to 2 years of age and 40–50 mL/kg/24 h in older children and adults. Making the diagnosis of conditions presenting with polyuria and polydipsia is difficult and management of DI is a challenge before the identification of the underlying cause [25–31].

Epidemiology

CDI is rare, with a reported prevalence of around 1 : 25,000 [20], and <10% of cases are hereditary [32]. The national surveillance of CDI in Denmark from an analysis of the registration of prescriptions for desmopressin to 1,285 patients over 5 years indicated a prevalence of 23 CDI patients per 100,000 inhabitants, with a higher prevalence in children and older adults [33]. The yearly incidence rate of new cases of CDI was 3–4 patients per 100,000 and the incidence of (presumably) congenital CDI was 2 infants per 100,000. X-linked NDI secondary to AVP receptor-2 mutations constitutes 90% of NDI cases, with a frequency of 4–8 per 1 million male live births and autosomal NDI accounts for the remaining 10% of cases [32]; abnormalities of the aquaporin-2 (*AQP2*) water channel gene, located on chromosome 12 at 12q13, are responsible for familial autosomal recessive and dominant forms of NDI.

The prevalence of Wolfram syndrome (**D**iabetes **I**nsipidus, **D**iabetes **M**ellitus, **O**ptic **A**trophy, and **D**eafness) has been variously reported between 1 and 9 per million (www.orpha.net). The frequency of autosomal dominant CDI due to AVP-neurophysin II (AVP-NPII) gene mutation is not known and genetic defects in AVP synthesis inherited as autosomal recessive or X-linked recessive traits are rare [31, 34].

Central Diabetes Insipidus

Increasing polyuria occurs when more than 80% of the AVP-secreting neurons are damaged. Extensive destruction can be caused by a variety of pathological processes including genetic causes. Autopsy studies after traumatic section of the pituitary stalk have revealed a loss of the large neurosecretory cells in the hypothalamic nuclei within 4–6 weeks; damage is greater after lesions at the level of infundibulum or above [21]. Patients with a familial form of diabetes insipidus show selective loss of magnocellular neurons in the PVN with moderate gliosis and relative preservation of small neurosecretory cells [35], suggesting that the disorder is due to degeneration of these hypothalamic neurons.

Aetiology

CDI in many patients is due to the destruction or degeneration of neurons originating in the supraoptic and PVN. The causes of these lesions include germ cell tumours, Langerhans cell histiocytosis (LCH), inflammatory/autoimmune conditions, vascular diseases, trauma resulting from surgery or an accident, metastases and midline cerebral and cranial malformations [25–31, 36–38]. Genetic defects in AVP synthesis inherited as autosomal dominant, autosomal recessive or X-linked recessive traits are rare [33].

X-linked (Xq28) NDI secondary to *AVPR2* mutations results in a loss of function or dysregulation of the renal AVP receptor-2 (V_2 receptor) [32]. Abnormalities of the *AQP2* (aquaporin 2) water channel gene located on chromosome 12 at 12q13 explain familial autosomal recessive and dominant forms of NDI (Table 14.2) [32].

Age at disease onset is shown in Figure 14.3: patients who did not have an intracranial tumour were significantly younger at diagnosis than those who did, and tumours were exceptional before age of 6 years [25].

Genetic Forms of Central Diabetes Insipidus

More than 60 mutations resulting in a defective prohormone and a deficiency of AVP have been described in familial neurohypophyseal CDI [13, 29, 33, 39–42]; all but a few have an autosomal dominant pattern of inheritance. Eleven patients, of whom 10 were from consanguineous families, have shown an autosomal recessive pattern of inheritance [43–46]. Despite some clinical similarities with the dominant form, the symptoms in these cases appear to be secondary to the reduced biological activity of the mutant AVP peptide. This hypothesis is supported by the high circulating concentration of mutant hormone, the absence of normal AVP hormone in the homozygous state and the absence of clinical or subclinical abnormalities in heterozygous carriers.

No mutations in the coding region, the intronic region or the 1.5-kb upstream region from the initial transcription site of the AVP-NPII gene were found in one Chinese family with an autosomal dominant inheritance pattern of overt CDI [47]. Linkage analysis indicated that the corresponding gene(s) responsible for the autosomal dominant form in this family was located in a 7-cM interval defined by two short tandem repeat markers on chromosome 20. This suggests locus heterogeneity of autosomal dominant CDI and implies genetic diversity in the cause of CDI. The autosomal dominant inheritance of this disease can occur through many mechanisms including dominant negative activity by interactions of mutant and wild-type (WT) precursors, accumulation of mutant precursor in the ER leading to stress protein response and autophagy and cellular toxicity by pathways that are still not completely defined. The study of the trafficking and processing of the mutant vasopressin prohormone *in vitro* has demonstrated that the mutation abolishes ER exit and processing of the vasopressin prohormone, resulting in an aberrant endoplasmic morphology and possible cell dysfunction and death. The presence of cytosolic autophagy suggests non-apoptotic cell death [48–50] but programmed cell death cannot be excluded [50].

Mutations involving the signal peptide decrease its ability to initiate proper processing of the prepro-AVP-NPII [46]; mutant precursors also impair intracellular trafficking of the WT precursor by forming heterodimers, thus reducing the bioavailability of active AVP by means of a 'non-toxic mechanism', i.e. a dominant negative effect [49, 51]. The demonstration of two pathways of degradation via the ER lumen and directly from the cytosol involving both the WT and the mutant prohormone suggests that the cytotoxic effect may result from processes that are quantitatively but not fundamentally different from those occurring in cells expressing the WT protein. Polyuria progressed in the absence of AVP neuronal loss in the knock-in mice expressing the mutant NPII that causes familial neurohypophyseal DI in humans, suggesting that cell death is not the primary cause of that disease. The aggregates accumulated in the ER and the cytoplasm of AVP cells might be involved in

Table 14.2 Aetiologies of diabetes insipidus.

Central diabetes insipidus	**Genetic** • Autosomal dominant/recessive (OMIM 125700), AVP mutation (OMIM 192340) • X-linked (OMIM 304900) • Congenital hypopituitarism with central diabetes insipidus (OMIM 241540) • Wolfram (DIDMOAD) syndrome (OMIM 222300), wolframin (WFS1) mutation (OMIM 606201) • Unknown genes (?) **Congenital** • Midline brain developmental defects – Septo-optic dysplasia – Holoprosencephaly – Absence of the internal carotid artery – Impairment of the neurohypophyseal arteries • Associated with ectopic posterior pituitary, anterior pituitary hypoplasia and congenital hypopituitarism **Acquired** • Idiopathic • Intracranial tumours – germinoma, craniopharyngioma, glioma • Langerhans cell histiocytosis • Autoimmune: lymphocytic hypophysitis/lymphocytic infundibulo-neurohypophysitis/ lymphocytic infundibulo-hypophysitis • Autoimmune (antibodies against vasopressin-producing cells, T-cell damage) • Granulomatosis (tuberculosis*, sarcoidosis*, Wegener*) • Congenital/postnatal infections/post-viral (varicella, congenital CMV and toxoplasmosis, encephalitis, meningitis) • Central nervous system surgery • Traumatic brain injury • Vascular impairment/hypoxic-ischemic • Metastases[a]/leukaemia relapse
Nephrogenic diabetes insipidus	**Genetic** • X-linked (OMIM 304800), AVPR2 mutation (OMIM 300538) (90%) • Autosomal recessive/dominant (OMIM 125800), AQP2 mutation (OMIM 107777) (10%) • Nephrogenic diabetes insipidus with mental retardation and intracerebral calcification (OMIM 221995) **Acquired** • Hypokalaemia, hypercalcaemia/hypercalciuria, alkalosis • Polycystic renal disease, others • Pyelonephritis, post-obstructive • Drugs
Primary polydipsia	• Psychogenic • Dipsogenic

[a] Rare in children.

the dysfunction of AVP neurons that lead to progressive polyuria, suggesting that dysfunction from accumulated mutant protein rather than direct neurotoxicity could be responsible for CDI [52, 53].

Early-onset CDI has been found to be associated with *de novo* mutations of the AVP-NPII gene and with hereditary Wolfram1 gene changes in three patients with early onset of polyuria and polydipsia, isolated AVP deficiency, no family history for CDI and normal brain MRI, with or without posterior pituitary hyperintensity [54]. The main findings appeared to be, on the one hand, the identification of two *de novo* dominant mutations of the AVP gene and, on the other, the description of a novel AVP mutation localized to the signal peptide (c.52_54delTCC). The majority of mutations reported thus far have been found in the part of the gene that encodes NPII, which is an intracellular binding protein for AVP, while only few mutations have been found in the signal peptide or in the AVP coding sequence and none in the region encoding copeptin (Human Gene Mutation Database).

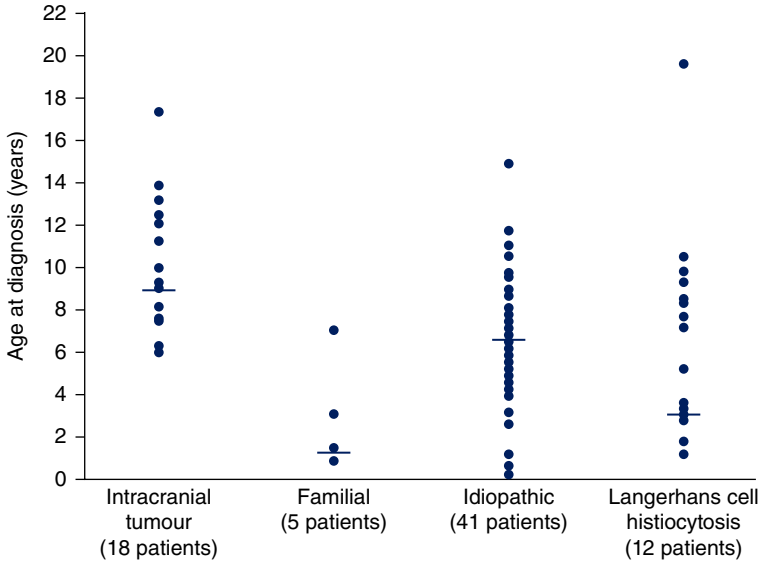

Figure 14.3 Age at disease onset and aetiology of central diabetes insipidus. *Source:* With permission from Maghnie et al. [25].

All the reported mutations replace or eliminate one or more amino acids known or reasonably assumed to be critical for proper processing, dimerization, disulphide bond formation and folding of the vasopressin precursor in the ER. In autosomal dominant CDI, abnormal processing of vasopressin precursors in the supraoptic and PVN and mutant AVP precursors impair intracellular trafficking of the WT precursor from the ER to the Golgi apparatus by forming heterodimers, with a reduction in the bioavailability of active AVP [49, 51, 52].

The loss of posterior pituitary hyperintensity on MRI can be explained by the abnormal processing of vasopressin precursors in the hypothalamic nuclei leading to the impairment of intracellular trafficking with a similar mechanism [29, 49, 51, 52]. In two patients, the signs and symptoms of CDI became evident by the age of 4 years, suggesting that both mutations are completely penetrant and that children with early-onset CDI must be evaluated for AVP mutations even in the absence of this condition among relatives. Although the most frequent form of familial CDI by far is the autosomal dominant condition [33], *de novo* dominant mutations of the AVP gene have been reported as recurrent mutations in three families [55].

Acquired Forms of Idiopathic Central Diabetes Insipidus (CDI)

Although 20–50% of cases are considered idiopathic, the identification of antibodies against vasopressin-secreting cells [28] and recent advances in imaging techniques have shed new light on CDI, making the idiopathic form very uncommon. Various clinical observations suggest an important role for autoimmunity in the pathogenesis of CDI. Indeed, autoimmune polyendocrinopathy and CDI associated with an MRI picture of thickened pituitary stalk suggest that patients with CDI and a thickened pituitary stalk may share a common aetiology [25, 56]. Circulating vasopressin-cell autoantibodies (AVPc-Abs) have been found in 75% of children and young adults with idiopathic CDI, suggesting that hypothalamo-neurohypophyseal autoimmune involvement is more common in children and young adults with idiopathic CDI than has generally been thought [57]. The higher frequency of AVPc-Abs in pediatric patients compared with the one-third found in adult patients with identical disease duration indicates that an autoimmune cause in idiopathic CDI is quite frequent. AVPc-Abs have been found in ~77% of subjects with combined posterior and AP dysfunction, a finding that goes well beyond the reported association of AP hormone defects in as many as 23% of subjects with isolated vasopressin deficiency. This indicates that AP involvement in the course of idiopathic CDI is highly suggestive of an autoimmune neurohypophyseal basis and fits well with the demonstration of infiltration of the pituitary stalk by lymphocytes [58].

In about a quarter of patients with idiopathic CDI, there is a temporal relationship between a viral infection (trigger) and the onset of CDI [25]. This hypothesis is strengthened by the fact that the pituitary gland is susceptible to CD8 T-cell-mediated autoimmunity triggered by a cell-specific model autoantigen [59] as well as by the possibility of inducing autoimmune hypophysitis by immunizing female SJL/J mice with mouse pituitary extracts [60]. The identification of AVPc-Abs in subjects who could have idiopathic CDI, LCH or a germinoma however indicates that this finding cannot be considered a completely reliable marker of autoimmune CDI [28]. Thus, to ensure a definitive diagnosis, close clinical and

MRI follow-up are needed because AVPc-Abs may mask germinoma or LCH.

The process underlying pituitary stalk thickening (PST) in 'idiopathic' CDI is not understood. Reports of a thickened pituitary stalk in association with autoimmune or inflammatory disease (lymphocytic hypophysitis [61, 62], necrotizing infundibulo-hypophysitis [61, 62] or lymphocytic infundibulo-neurohypophysitis) [63] focus on adults with histological features of lymphocyte and plasma cell infiltration, fibrosis and necrosis. Lymphocytic hypophysitis is thus a rare chronic inflammatory process that affects the pituitary gland variably. It is worth emphasizing that clear-cut criteria for the diagnosis of lymphocytic hypophysitis in children and adolescents are lacking and that CDI is present in only about 20–25% of patients with a thickened stalk.

The term lymphocytic infundibulo-hypophysitis has been coined [25] to distinguish children and adolescents with CDI, AP hormone deficiency, reduction of AP size and transient or persistent pituitary stalk (PS) thickening from adult patients with similar posterior pituitary (PP) and PS findings at MRI but with normal AP size and function [61]. GHD was defined in these adult patients as a GH response after pharmacological stimulation tests lower than 10 µg/L and not 3 µg/L. In such adult cases, the term lymphocytic infundibulo-neurohypophysitis is more appropriate [61].

Vascular CDI

CDI may be caused by vascular brain damage but the pathophysiology of such a mechanism is not understood. In one group of patients with idiopathic CDI and normal AP function, standard MRI showed normal PS and AP gland size [26] and dynamic MRI studies after contrast medium injection demonstrated the absence of posterior pituitary lobe enhancement, whereas normal enhancement of the AP was present. The lack of contrast enhancement of the posterior lobe suggested that a selective vascular injury to the inferior hypophyseal arteries could be causally linked to CDI. The mechanism affecting posterior pituitary blood supply remains undefined but the possibility that a congenital lack or poor development of the posterior pituitary vascular system without evidence of macroscopic morphological abnormality of the pituitary gland on MRI or secondary changes of vascular supply due to a local inflammatory process cannot be excluded.

Langerhans Cell Histiocytosis (LCH)

CDI is the most frequent CNS manifestation of LCH, occurring in 10–50% of all patients [64, 65]. The risk of developing CDI after diagnosis and specific therapy has been shown to be 16% at 5 years and 20% at 15 years, which is typical of a multisystem disease followed by lesions in the craniofacial area [64].

Some patients with CDI and endocrinopathies seem to be at risk for neurodegenerative CNS disease although brain and pineal involvement can be diagnosed shortly after the onset of CDI [66]. Growth hormone deficiency (GHD) is the most frequent additional deficit accounting for 42% of cases with CDI and LCH. The 10-year cumulative incidence of GHD in a French LCH survey was ~54% among patients with CDI [67]. The identification of circulating AVPc-Abs in LCH patients and their tendency to spontaneous clearance [28, 56] suggest that these autoantibodies might be an LCH-related immune epiphenomenon.

PST can be found in ~50–70% of patients with LCH at presentation or at follow-up [25, 68] and may be present before CDI onset. AP size has been found to be normal, reduced or rarely enlarged [25, 64, 69]. A search for extra-cranial lesions suggestive of LCH by dermatological and bone surveys, chest X-ray and ear, nose and throat examination in patients with PST is recommended and could reduce the need for intracranial biopsies.

Sarcoidosis

Sarcoidosis is a multisystem disease of unknown aetiology; the central nervous system is involved in 5–15% [70] of patients and precedes other symptoms in 25–30% of cases [71, 72]. Autopsy studies have demonstrated that sarcoid granulomata have a predilection for the hypothalamus and involve the PS or the pituitary gland less commonly [71]. Thus, patients with neuroendocrine sarcoidosis commonly have hypothalamic dysfunction with hypothalamic disturbances and AP hormone deficiency. Polyuria/polydipsia is the most common symptom reported in 25–33% of adult patients affected by neurosarcoidosis and endocrinopathy is relatively rare [70, 72]. In pediatric patients, hypophyseal dysfunction was present in 21% of cases, 66% of whom were affected by CDI. Children with neurosarcoid present differently than adults and are more likely to have seizures and less likely to have cranial nerve palsies; eye disease such as uveitis may occur in younger children [73].

Brain MRI studies show heterogeneous features, including periventricular white matter foci, leptomeningeal enhancement, hydrocephalus and enlargement of the PS [72], the latter entity being described in four of the five patients reported by Bullmann et al. [71]. Only a few pediatric cases of CDI secondary to neurosarcoidosis have been described [72].

Germinomas

Intracranial germ cell tumours comprise 8% of primary pediatric brain tumours. MRI findings suggest that suprasellar and neurohypophyseal germinomas arise from the posterior pituitary to the infundibulum [74]. Partial or complete stalk thickening is detectable in

78–100% of cases at presentation and may be the only finding in small germinomas [74]; its presence increases the risk of malignancy to about 15–17%, while the risk decreases to 3% in patients with a normally sized stalk.

Serial contrast-enhanced brain MRI in patients affected by CDI with PST (every 3–6 months for the first 2 years) may reduce the amount of time for diagnosis of germinoma by a year [25] but a thickened stalk has been reported up to 5 years after the onset of CDI and preceded by a lymphocytic tissue infiltration as a host reaction to the presence of a germinoma that could mask the diagnosis [75]. Occasionally, germinoma can mimic multisystemic LCH with vertebral compression, recurrent ear infections, thickened PS, enlarged pineal gland and negative serum and cerebrospinal fluid (CSF) germ cell tumour markers, as demonstrated in a 9-year-old girl [76].

The role of hCG and other tumour markers in the early diagnosis of germinoma is not well understood. A negative result in the CSF does not exclude a germinoma [25]. The presence of circulating AVPc-Abs in these patients before treatment [28] can mask the diagnosis of germinoma. Pituitary stalk biopsy is mandatory in the presence of progressive thickening to more than 6.5–7 mm and/or AP enlargement. Growth arrest and multiple pituitary hormone deficiency are common and are usually early findings in pituitary germinomas (almost 100% of cases at follow-up). Hormone deficiency is not predictive of its presence.

Craniopharyngioma and Post-Surgical CDI

Craniopharyngioma is a benign tumour arising from squamous cell nests in Rathke's pouch. It constitutes 6–9% of all intracranial tumours in children and is the most frequent suprasellar neoplasm in the pediatric population (54% of cases) [27]. Presentation includes visual impairment due to compression of the optic chiasm and bilateral optic atrophy; systemic symptoms relate to raised intracranial pressure in 60–75% of cases. In various large pediatric series, signs and symptoms of AP dysfunction were detected in about 20–70% of cases [27]. CDI and multiple pituitary hormone deficiency are common complications. The frequency of CDI before surgery varies from 16 to 55%, while permanent CDI after surgery accounts for up to 80% of cases; transient CDI is reported in 13% of cases [77].

Impairment of hypothalamic–posterior pituitary function after complete section of the PS is a common, predictable outcome characterized by the classic triphasic response of urine volume. The initial diuretic phase (1–4 days) is followed by a second phase of oliguria, which may reflect degeneration and death of neurosecretory neurons with release of stored AVP into the circulation for 4–7 days. Permanent CDI follows.

The diagnosis of CDI after surgery is often made within a few hours, although abnormalities of AVP secretion and fluid balance often begin during the intraoperative period [77]. The transsphenoidal approach now widely used for both pituitary and some suprasellar tumours is associated with a lower incidence of postoperative CDI. CDI after a trans-frontal approach has been reported in association with high plasma vasopressin (AVP) immunoreactivity but the plasma showed no antidiuretic bioactivity or antidiuretic response to standard AVP was greatly attenuated, suggesting the presence of a circulating vasopressin antagonist affecting the renal action of endogenous and exogenous AVP. This finding has not been confirmed by other studies.

Metastasis

Metastasis to the posterior pituitary is well known in disseminated cancer due to the direct arterial vascularization of the posterior pituitary lobe. The incidence of pituitary metastases varies from 0.14 to 28.1% of all brain metastases and is higher in adult autopsy series [78]. They most frequently originate from lung, breast and gastrointestinal carcinomas and from leukaemia/lymphoma with symptoms seen particularly in terminal stages [79]. About 20% of metastases to the pituitary–hypothalamic axis are diagnosed clinically and CDI is the main presenting symptom. A review of the literature showed that CDI has been reported in association with leukaemia in 39 of 5778 children (0.6%), 4 of whom were under 10 years of age [79].

Destructive and non-homogeneously enhancing intrasellar and suprasellar lesions and involvement of adjacent structures can be observed on MRI; the pituitary stalk can be involved and appears entirely or partially thickened. Progressive thickening of the PS has been the presenting symptom in various pediatric cases of primary lymphoma of the central nervous system or of myeloid leukaemia. CDI and multiple AP hormone deficiencies can precede by one or more years the diagnosis of malignancy [80, 81].

Other Entities

CDI has been reported in Wegener granulomatosis, a disease characterized by necrotizing vasculitis and granulomatous inflammation of the upper and lower respiratory tract together with glomerulonephritis [82]; MRI showed an isointense suprasellar mass and enlargement of the infundibulum. Two months after corticosteroid treatment, MRI showed nearly complete resolution of pituitary lesions and dramatic clinical improvement.

Transient CDI associated with tuberculosis is well known. Tuberculosis of the CNS is the most serious complication in children, accounting for about 3–4% of untreated tuberculosis infections in developed countries [83]. It usually arises from a metastatic caseous lesion in the cerebral cortex or meninges that develops during lymphohaematogenous dissemination of the primary

infection. A few reports refer to tuberculous meningitis followed by acute onset of CDI variably associated with seizure and/or communicating hydrocephalus; in these cases, MRI showed PST [84]. Other infective causes include meningitis due to, for example, group B streptococcus.

Acute post-traumatic CDI has been reported in 22 of 85 patients who suffered a moderate to severe traumatic brain injury [85]; five of these patients had persistent abnormal water deprivation tests at a median time of 17 months from TBI and expression of permanent partial CDI; the remaining patients had complete recovery of PP function. In this study [85], permanent CDI correlated with lower Glasgow Coma Scale but not with age, gender, basal skull fracture or operative mass evacuation. Post-traumatic DI may result from inflammatory oedema around the hypothalamus or posterior pituitary with resolution as the swelling resolves. It can also result from direct damage to the paraventricular and supraoptic hypothalamic neurons, the pituitary stalk or axon terminals in the posterior pituitary [85].

Diagnosis of Central Diabetes Insipidus

Clinical Manifestations

Clinical examination may provide important pointers to underlying diagnoses [29]. The age at which symptoms develop and the pattern of fluid intake may influence subsequent investigation. The primary symptoms are persistent polyuria and polydipsia; young children may have severe dehydration, vomiting, constipation, fever, irritability, sleep disturbance, nocturia, failure to thrive and growth retardation. Severe dehydration in males is suggestive of NDI; some mental deterioration probably caused by chronic and unrecognized dehydration has been reported.

In a large cohort of patients with CDI of different aetiologies [25], 40% of patients displayed symptoms other than polyuria and polydipsia at presentation; headache was common, but not particularly associated with intracranial tumour, whereas visual defect was. Growth retardation was not more common in patients with CNS tumours, which contrasts with previous reports indicating that such delays strongly suggest an intracranial tumour. Patients who did not have an intracranial tumour were significantly younger than those who did and none of the patients with intracranial tumour were younger than 6 years of age (Figure 14.3). Careful attention to other signs such as recurrent otitis media, skin lesions, cholangitis, dyspnoea or bone pain/lesions is needed in order to exclude multi-organ involvement by LCH.

In autosomal dominant CDI, onset of the disease ranges from the first to the sixth year of life but cases of earlier or later onset have also been reported [86]. Symptoms in patients with early onset of mild polyuria and polydipsia usually worsen with time, especially before ten years of age, but it is also possible that complete CDI is expressed from the neonatal period [37, 69]. In our cohort of 8 families, the median age of onset of polyuria and polydipsia was 27 months (range of 12–72; unpublished data).

The wide variability in the age of onset and the severity of the AVP deficiency among patients with the same mutation may be ascribed to individual differences among such patients, like the rate of production of the mutant precursor, the intensity of neurohypophyseal stimulation, the individual susceptibility to the toxic effect of the mutant precursor, the ability to degrade mutant precursors and the variations in secretory reserve capacity or in the development of the gland itself.

In patients with Wolfram syndrome 1, the frequency of CDI varies from 48 to 78% [87, 88]. Diabetes mellitus has been described as the usual first symptom presenting at a median age of 6 years, followed by the onset of optic atrophy at 11 years [89, 90]. The development of polyuria and/or enuresis can indicate diabetes insipidus and the time of onset varies considerably, not generally appearing until the second or third decade [68, 91]. CDI may initially be partial. A diagnostic flow chart for the diagnosis of CDI is shown in Figure 14.4.

Baseline Laboratory Workup

The diagnosis of CDI can be straightforward but limitations and poor accuracy of biochemical tests for the differential diagnosis of polyuria and polydipsia can lead to incorrect management [31, 92].

The first step is to establish polyuria and polydipsia; polyuria is defined by urine volume >2 L/m^2/24 h or by 150 mL/kg/24 h at birth (> 6 mL/kg/h), 100–110 mL/kg/24 h up to 2 years (>4 mL/kg/h) and 40–50 mL/kg/24 h in the older child and adult. 24 hour evaluation of water balance is mandatory and the presence of polyuria in the absence of solute diuresis (glucose, calcium) should raise the suspicion of diabetes insipidus [28].

In infants or young children, random morning plasma osmolality after discontinuing water intake at midnight >295 mOsm/kg H_2O and/or serum sodium >143–145 mmol/L with urine osmolality <300 mOsm/kg or urine/plasma osmolality ratio <1 are diagnostic of diabetes insipidus.

The accuracy of measuring plasma osmolality in routine hospital laboratories by freezing point depression is usually not good enough to fulfil the quality criteria required (coefficient of variation of 1% or less at 290 mOsm/kg H_2O), especially when osmolality is determined in serum or frozen plasma. When an osmometer is not available, a good estimate of plasma osmolality, usually accurate to within 1–3% (i.e. within 9 mOsm/kg

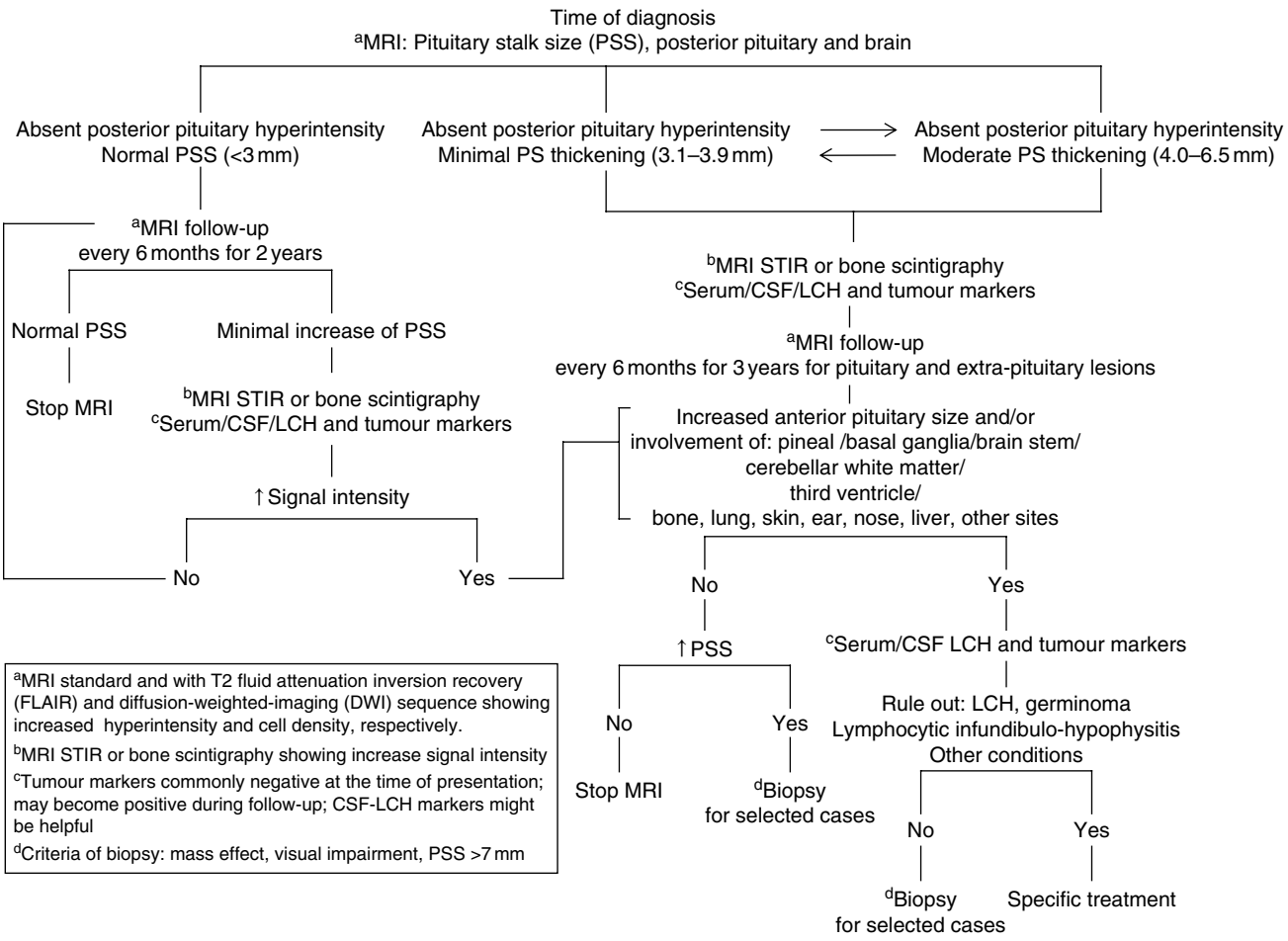

Figure 14.4 Central diabetes insipidus: clinical and radiological follow-up algorithm.

H_2O) of what is determined directly by osmometry [22], can be obtained using the formula

$$\mathrm{POsm} = 2\left[\mathrm{Na}+\right] + \frac{\mathrm{glucose}\left(\mathrm{mg/dlL}\right)}{18} + \frac{\mathrm{BUN}\left(\mathrm{mg/dlL}\right)}{2.8},$$

where 18 and 2.8 represents the molecular weights of glucose and blood urea nitrogen (BUN), respectively, if originally expressed in mg/dL.

Water Deprivation Test and DDAVP Challenge

In the absence of a straightforward diagnosis, a water deprivation test and 1-deamino-8-D-arginine vasopressin (DDAVP) trial are needed [93–95]. Close collaboration must be arranged with the laboratory in advance in order to ensure prompt reporting of results.

A 7 hours (or less) deprivation test is usually sufficient, except in cases of primary polydipsia when a longer period of fluid deprivation may be required. The patient must be under continuous surveillance and the test be discontinued if weight loss exceeds 5% of starting weight and/or plasma sodium concentration is higher than 143–145 mmol/L and/or plasma osmolality is higher than 295–300 mOsm/kg H_2O and/or urine osmolality increases to normal. The measurement of plasma AVP after a deprivation test [92] or hypertonic saline infusion [38] is not helpful.

Although measurement of plasma copeptin, either at baseline or during different tests, has been proposed as a reliable stable surrogate for plasma AVP [27, 96], diagnostic cut-off values are still to be validated. Urinary AQP2 was described for the differential diagnosis of CDI versus NDI 20 years ago [97] but its use in daily practice is very limited.

The administration of 5–10 µg of desmopressin (DDAVP) intranasally will help differentiate CDI from NDI. Alternatively, the desmopressin can be given subcutaneously or orally, provided that appropriate dose adjustments are made.

Tumour Markers

Once the diagnosis of CDI has been established, germ cell tumour markers including serum and CSF human chorionic gonadotropin, placental alkaline phosphatase

and alpha-fetoprotein should be sought. They may be negative at presentation in patients with CDI and pituitary stalk involvement but may develop over time [98].

Vasopressin-Secreting Cell (AVPc-Abs) and Pituitary Antibodies

While the identification of antibodies against vasopressin-secreting cells (AVPc-Abs) and pituitary antibodies has shed new light on the pathogenesis of CDI [4, 99–101], the diagnostic role of AVPc-Abs remains questionable [28]. The identification of AVPc-Abs in subjects with idiopathic CDI, LCH or germinoma indicates that this finding cannot be considered a reliable marker of autoimmune CDI but rather a surrogate of immune response [28]. Thus, to ensure a definitive diagnosis, clinical and MRI follow-up are needed since the presence of AVPc-Abs may mask germinoma or LCH.

Autoimmune polyendocrinopathy and CDI associated with an MRI picture of thickened pituitary stalk [25] as well as the histological diagnosis of lymphocytic infundibulohypophysitis associated with CDI [58, 63] suggest that such patients may share a common aetiology [25, 56, 102]. The fact that the pituitary gland is susceptible to CD8 T-cell-mediated autoimmunity triggered by a cell-specific model autoantigen [59], as well as to the development of autoimmune hypophysitis through the immunization of female SJL/J mice with mouse pituitary extracts, supports this theory [60]. In other words, two different potential pathogenetic mechanisms for the induction of primary hypophysitis have been identified: one directed against self-antigens (T helper dominance) and the other against non-self-antigens (postinfection) [25, 103].

The reduction of AP size over time in patients with CDI and self-limited pituitary stalk thickness suggests an inflammatory/autoimmune condition as compared with patients with germinoma [25, 30]. Likewise, lymphocytic hypophysitis, where a swelling of the pituitary gland at presentation was followed by pituitary atrophy and empty sella, has been demonstrated in murine models immunized with pituitary proteins [104].

Genetic Testing

A novel and recurrent mutation in AVP-NPII gene has been reported in three families [55] and early-onset CDI has been recently found to be associated with *de novo* mutations of the AVP-NPII gene and with hereditary Wolfram1 gene changes in three patients with early onset of polyuria and polydipsia, isolated AVP deficiency, no family history for CDI and normal brain MRI, with or without posterior pituitary hyperintensity [54].

Imaging Techniques

Magnetic resonance imaging (MRI) with high field systems is the examination of choice for imaging the hypothalamic–pituitary axis. Standard evaluation includes spin-echo (SE) T1-weighted images and turbo/fast spin-echo (TSE) T2-weighted images on sagittal and coronal planes with a slice thickness of 2–3 mm. Post (gadolinium chelate)-contrast imaging with T1-weighted sequences should always be performed on sagittal and coronal planes. Heavily T2-weighted images (i.e. driven equilibrium [DRIVE], constructive interference in steady state (CISS) or fast imaging employing steady-state acquisition [FIESTA] sequences) acquired on the sagittal plane at submillimetric thickness are highly recommended since they provide more detailed information on the suprasellar compartment because of the excellent contrast between CSF and the adjacent parenchymal structures [105]. We also always perform a fluid attenuation inversion recovery (FLAIR), diffusion-weighted imaging (DWI) and post-contrast T1-weighted sequence of the whole brain on the axial plane to screen for additional brain abnormalities. Among advanced MR techniques, MR spectroscopy (MRS) may be used in selected cases for the evaluation and characterization of sellar–suprasellar mass lesions [106].

A complete neuroradiological evaluation including axial DWI, FLAIR and post-contrast T1 covering the whole brain, along with pre-contrast sagittal T1, coronal T2, sagittal T2-DRIVE or equivalent and post-contrast sagittal and coronal T1-weighted imaging focused on the sellar region requires ~30 minutes and is the recommended baseline study for all patients with CDI (Table 14.3). MRS may be acquired on a case-by-case basis.

Additional imaging tests including spinal MRI and/or whole body radiological scanning can be required. Spinal MRI should be performed when mass lesions have the potential to disseminate or to rule out vertebral involvement. Standard whole body imaging includes a skeletal survey and chest X-ray. Positron emission tomography (PET) with 18F-fludeoxyglucose (18F-FDG) or amino acid analogues and whole body MRI that allows imaging of the entire body with one or multiple sequences in a single examination can also be useful. For extra-pituitary-associated lesions, the most commonly used sequence in whole body MRI is a short tau inversion recovery (STIR) in the coronal plane. These techniques are being evaluated and, although MRI STIR is particularly attractive for examining children because of its wide availability and lack of radiation exposure, they are still not considered alternatives to the standard skeletal survey [31, 107].

Imaging Findings in CDI

Neuroimaging findings in patients with CDI typically consist of a lack of visualization of the posterior pituitary bright spot and thickening of the pituitary stalk including the proximal (>3.0 mm), distal (>2 mm) or entire stalk

Table 14.3 Neuroradiological protocol in CDI.

Baseline study	Sagittal SE T1	Anterior pituitary morphology, height and length. Bright spot evaluation. Haemorrhagic/high protein concentration lesions
	Sagittal T2-DRIVE	Baseline and follow-up pituitary stalk evaluation. Optic chiasm and nerves morphology. Pineal gland evaluation. Depiction of the relationship between suprasellar mass lesions and surrounding structures
	Coronal TSE T2	Pituitary width and shape evaluation. Pituitary stalk orientation. Optic chiasm depiction
	Axial FLAIR	Screening sequence to evaluate the whole brain. In children below the age of 2 years, instead of axial FLAIR, axial T2-weighted image is recommended
	Axial DWI	Screening sequence. Estimation of differences in cell density and tissue structure (i.e. sellar–extrasellar mass lesions)
	Coronal, sagittal and axial post-contrast T1	Pituitary stalk evaluation. Mass lesion characterization. Secondary dissemination
Complementary study (performed on a case-by-case basis)	MRS	Detection and estimation of normal and abnormal brain metabolites (i.e. sellar–suprasellar mass lesion characterization)
	Spine MRI	Secondary leptomeningeal dissemination (germinomas). Vertebral involvement (LCH)

SE, spin echo; DRIVE, driven equilibrium; TSE, turbo spin echo; FLAIR, fluid attenuation inversion recovery; DWI, diffusion-weighted imaging; MRS, magnetic resonance spectroscopy.

(Figure 14.5) but this finding is not specific because it can be found in patients with germinoma, histiocytosis, lymphocytic hypophysitis or much rarer secondary causes (e.g. sarcoidosis, lymphoma, etc.). It can also be present in idiopathic cases [108].

Persistence of the posterior pituitary hyperintense signal suggests familial CDI. In autosomal dominant CDI, the identification of posterior pituitary hyperintensity does not necessarily indicate that the functional integrity of the hypothalamic–neurohypophyseal axis is preserved [29] and, even if it is present initially, the signal disappears at follow-up. Since the neuroimaging findings lack specificity, a search for extrasellar lesions is strongly recommended to direct the aetiological diagnosis.

Involvement of the pineal gland or basal ganglia is highly suggestive of a germinoma. Synchronous lesions in the hypothalamic and pineal regions (Figure 14.6) account for about 10% of all intracranial germ cell tumours (so-called bifocal germinomas). Germinomas on MRI are generally isointense to grey matter on T1-weighted images and iso- to hypointense on T2-weighted images. Contrast enhancement is usually moderate to marked. Calcification and cystic–necrotic changes are rare [109].

DWI may show restricted diffusion reflecting the highly cellular lymphocyte component within the tumour (Figure 14.7), while proton MRS is characterized by predominance of choline peak, residual creatine peak, absence or marked reduction of NAA and possible lactate and lipid peaks [110]. DWI is helpful in discriminating germinomas from other common paediatric suprasellar neoplastic lesions such as craniopharyngiomas and gliomas but it is unable to confidently differentiate suprasellar germinomas from lymphocytic infundibulo-hypophysitis or LCH. In the case of non-specific MRI findings, additional information can be gathered by PET imaging with amino acid analogues such as 11C-methionine (MET). MET-PET has been reported to be very useful for the detection of basal ganglia germinomas without overt mass formation for locating a precise biopsy target and in monitoring treatment [111, 112]. Germinomas are prone to disseminate with subependymal or leptomeningeal enhancement (Figure 14.7). In such cases, evaluation of spinal MRI is mandatory.

The search of extrasellar lesions can also be helpful to point a diagnosis of LCH. Brain MRI can reveal subcutaneous soft tissue or skull lesions typical of this disorder (Figure 14.8). In such cases, brain CT can help define bone involvement better and reveal additional lesions. If LCH is suspected, the search for extra-cranial lesions (bone survey, chest X-ray) is also mandatory and can significantly reduce the need for intracranial biopsy.

Newer imaging modalities such as whole body MRI and 18F-FDG PET have been compared with plain radiography and bone scintigraphy and have greater accuracy [113, 114]. The diagnostic value of 18F-FDG PET has also been compared with MRI in patients with LCH

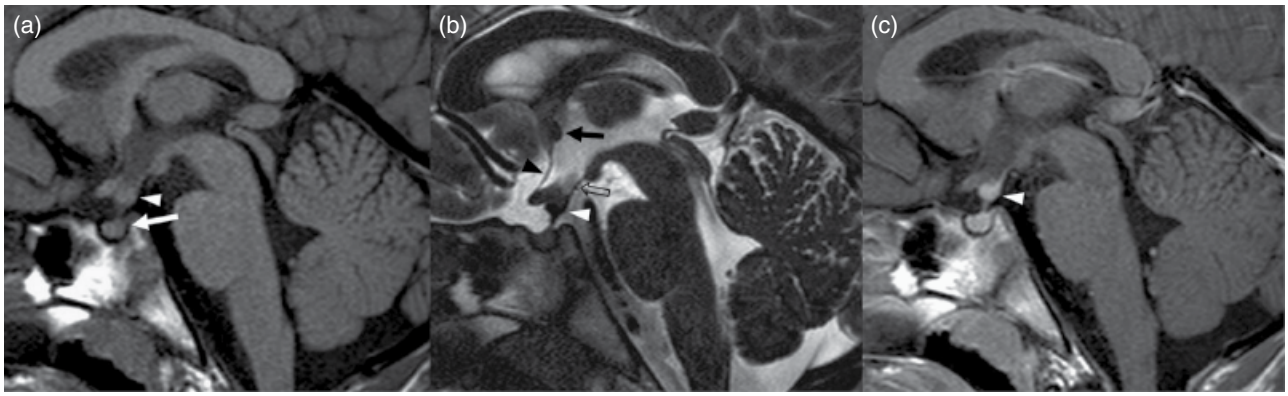

Figure 14.5 Central diabetes insipidus and growth hormone deficit in an 11-year-old boy. (a) Sagittal T1-weighted image. (b) Sagittal T2-DRIVE image. (c) Gd-enhanced sagittal T1-weighted image. The posterior pituitary lobe is not visualized (white arrow, a). There is pathologic thickening of the median eminence and proximal pituitary stalk (white arrowheads, a–c). The distal pituitary stalk is barely visible on pre-contrast T1-weighted image (a), whereas it is clearly demonstrated on T2-DRIVE (b) and post-contrast (c) images. Notice the sharp delineation of the suprasellar compartment on T2-DRIVE image that allows optimal depiction of the floor of the third ventricle (black open arrow, b), lamina terminalis (black arrowhead, b) and anterior commissure (black arrow, b).

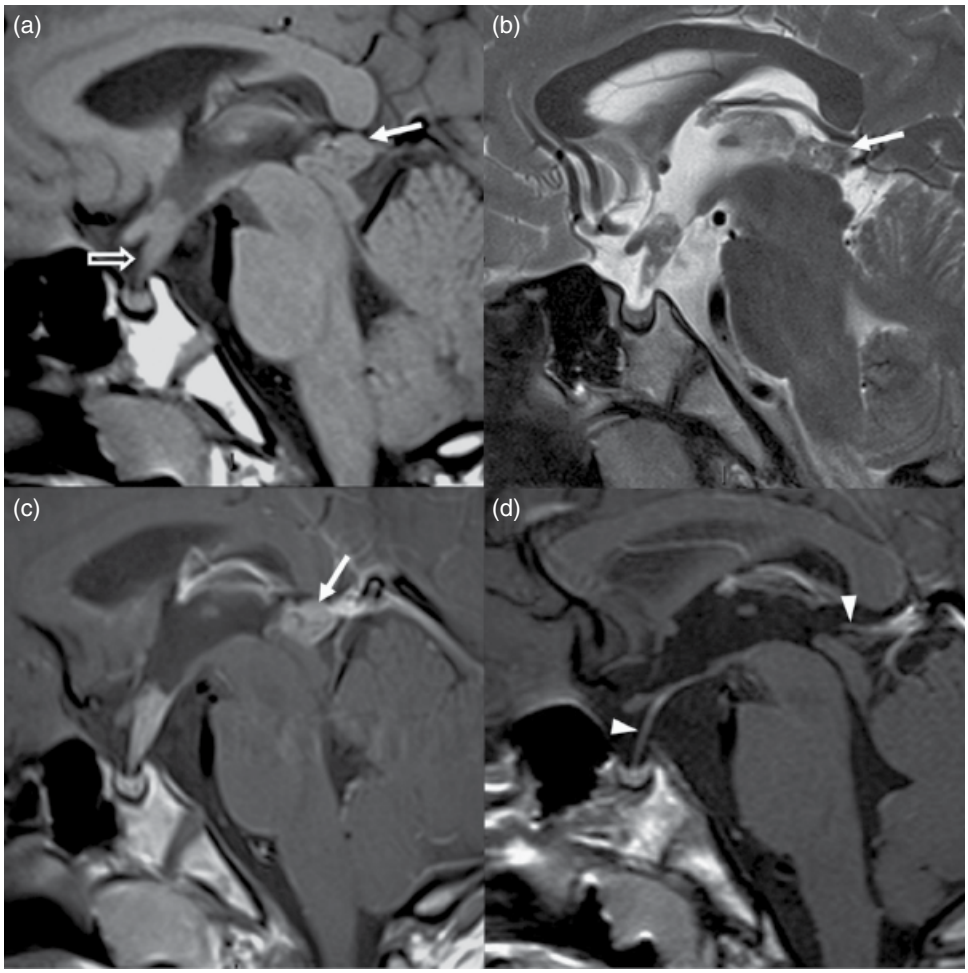

Figure 14.6 Bifocal germinoma. (a) Sagittal T1-weighted image. (b) Sagittal T2-weighted image. (c, d) Gd-enhanced sagittal T1-weighted images. Absent bright spot (a) and thickening of the pituitary stalk (open arrow, a). There is also pathologic involvement and mild enlargement of the pineal gland (arrows, a–c) in keeping with a bifocal germinoma. Follow-up MRI performed 6 months after chemotherapy shows complete resolution of the picture (arrowheads, d).

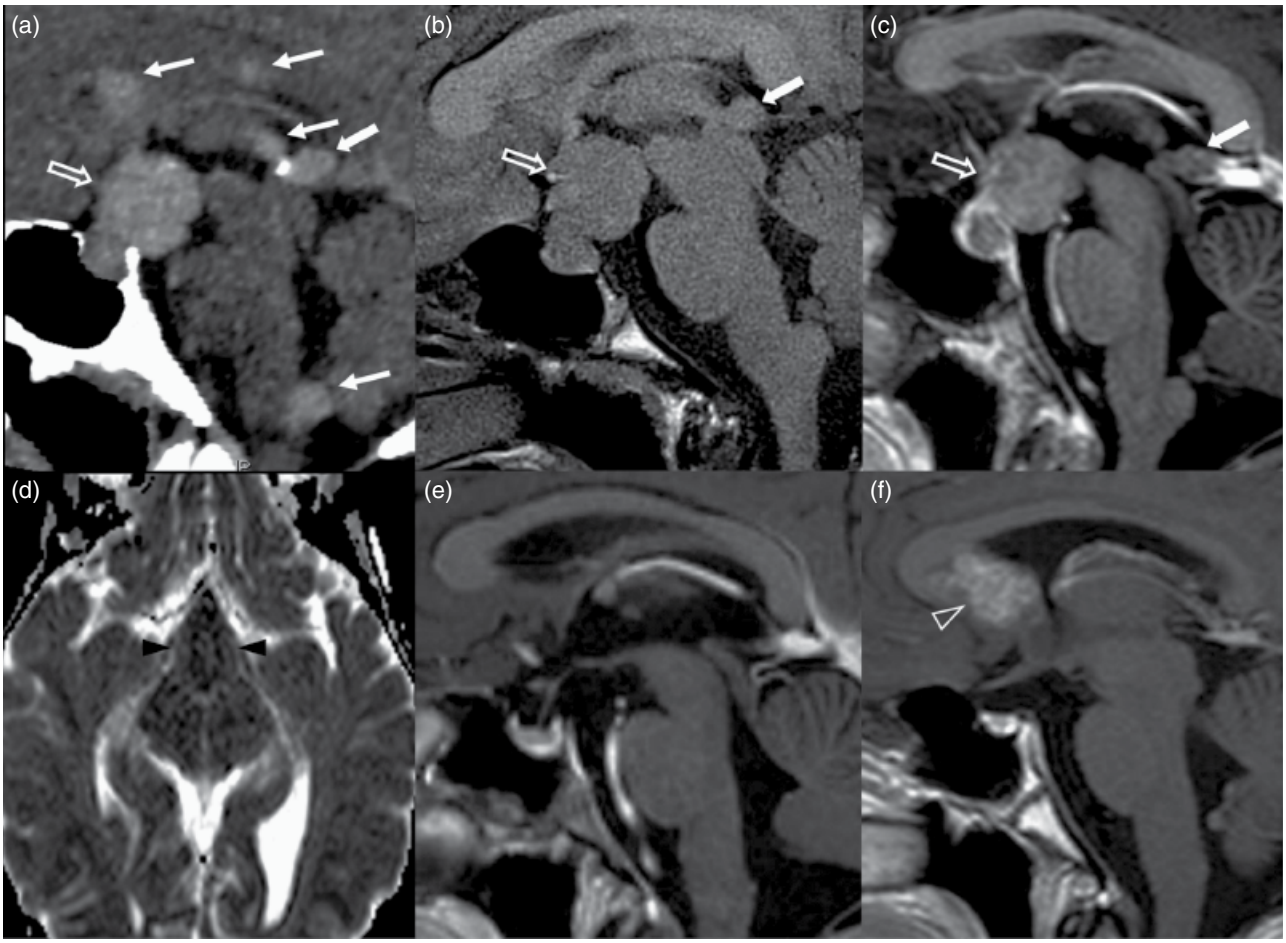

Figure 14.7 Metastatic germinoma remission and relapse in a 10-year-old girl. (a) Sagittal CT image. (b) Sagittal T1-weighted image. (c) Gd-enhanced sagittal T1-weighted image. (d) Axial apparent diffusion coefficient (ADC) map. (e,f) Follow-up, Gd-enhanced sagittal T1-weighted images. Neuroimaging studies at admission show a solid mass of the hypothalamic–hypophyseal region (open arrows, a–c) demonstrating a compact structure and reduced diffusivity (black arrowheads, d) in keeping with increased cellularity. There is concomitant pathologic involvement of the pineal gland (thick white arrows, a–c). Secondary lesions, better visible on the sagittal CT image (thin white arrows, a), demonstrate similar density of the primitive lesion (spontaneous hyperdensity). Follow-up MRI at 1 year, following chemotherapy and radiotherapy, shows complete resolution of the picture (e); subsequent MRI study performed 2 years later shows distant disease relapse in the subependymal frontal region (open arrowhead, f).

for primary staging and disease follow-up. 18F-FDG PET was more accurate in evaluating disease activity after chemotherapy but the overall sensitivity of MRI was superior to that of PET [115].

The detection of focal or confluent areas of T2/FLAIR hypersignal that typically involve the cerebellar white matter and dentate nuclei, sometimes the pontine tegmentum and, occasionally, the basal ganglia (Figure 14.9), is an additional extrasellar diagnostic clue suggestive of LCH. These lesions represent the second most common intracranial manifestation of LCH and have been interpreted as indicative of a neurodegenerative process, since histopathologic examination revealed neuronal loss and axonal degeneration together with profound T-cell inflammation [116]. A high frequency of pineal cysts and enlarged pineal glands has been reported in patients with LCH. This finding is non-specific and may reflect direct pineal infiltration by LCH or hyperplasia of the gland [117].

Additional and extremely important diagnostic information can be obtained by serial imaging. Concomitant volumetric increase in the size of the stalk and AP on follow-up supports the diagnosis of infiltrative/neoplastic disorders, particularly germinoma and exceptionally LCH. On the other hand, the association of AP hormone deficiency with MRI evidence of progressive reduction in size of the AP is suggestive of an inflammatory cause such as lymphocytic infundibulo-hypophysitis [25, 30] (Figure 14.10).

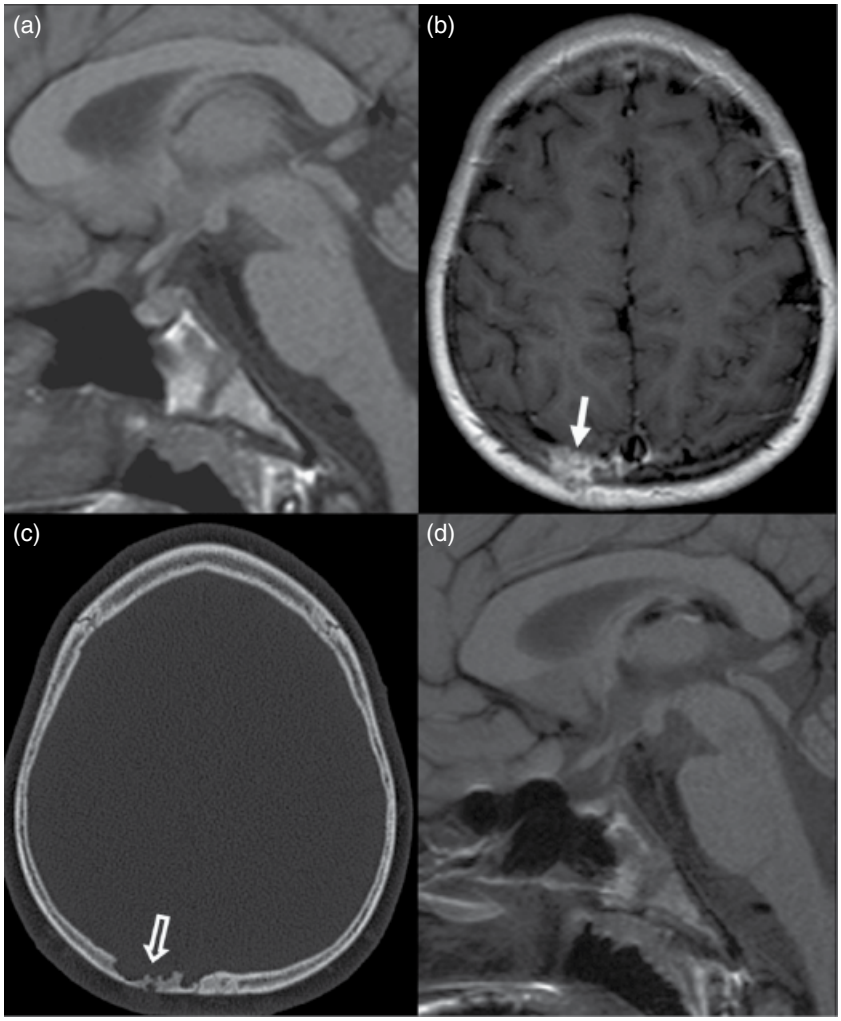

Figure 14.8 Langerhans cell histiocytosis. Pre-contrast sagittal T1-weighted image (a) performed at admission shows absent bright spot, mild enlargement of the pituitary gland and normal pituitary stalk. Brain survey with post-contrast axial T1-weighted image reveals a focal lesion involving the right parietal bone with marked enhancement (arrow, b), suggesting a diagnosis of Langerhans cell histiocytosis. Brain CT confirms a lytic lesion of the right parietal bone (open arrow, c). Post-treatment 1 year follow-up sagittal T1-weighted image shows reduction in size of the pituitary gland (d).

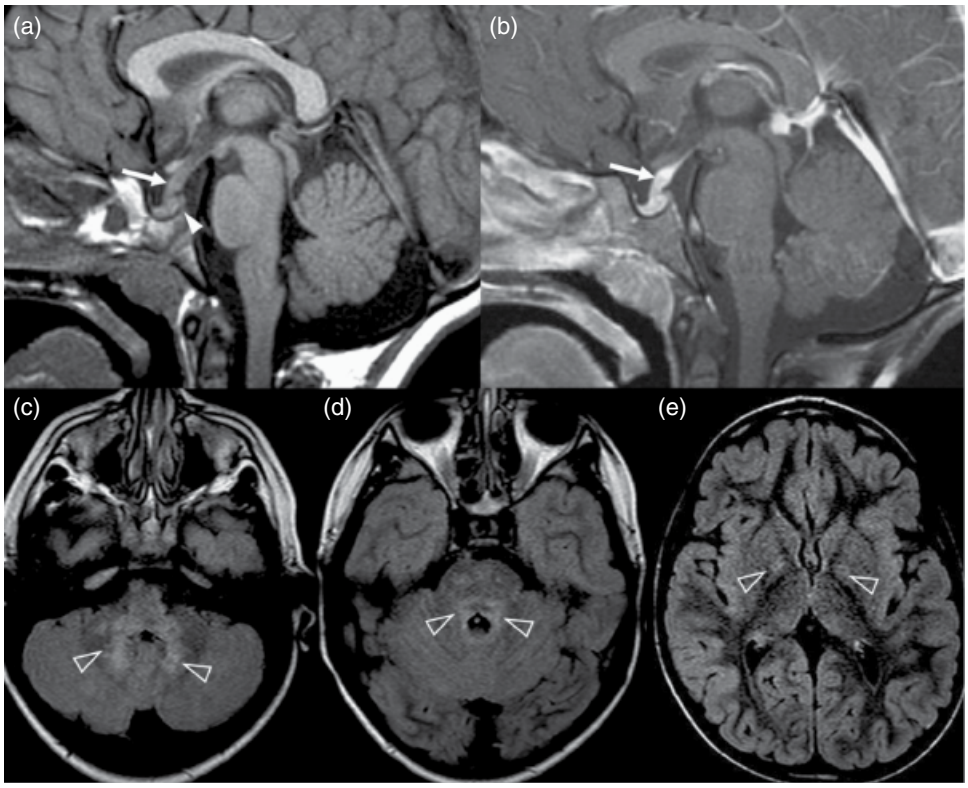

Figure 14.9 Langerhans cell histiocytosis with CNS involvement. (a) Sagittal T1-weighted image. (b) Gd-enhanced sagittal T1-weighted image. (c–e) Axial FLAIR images. The posterior bright spot is not visualized (arrowhead, a). The pituitary stalk is thickened (arrows, a,b). Ill-defined areas of FLAIR hyperintensity involve the brain stem, the dentate nuclei region and the globus pallidus bilaterally (open arrowheads, c–e) in keeping with degenerative changes.

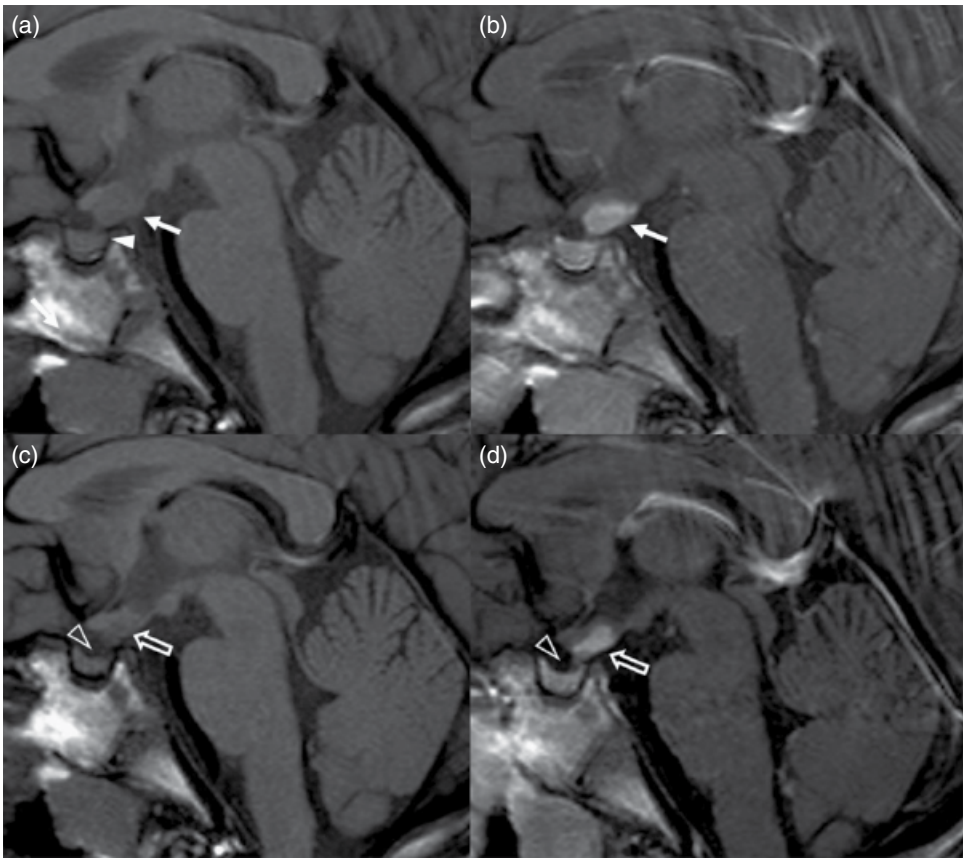

Figure 14.10 CDI: Evolution of findings in lymphocytic infundibulo-hypophysitis. (a, b) Baseline brain MRI. Pre- and post-contrast sagittal T1-weighted images show absent bright spot (arrowhead, a) and thickened pituitary stalk (arrows, a,b). (c, d) Follow-up MRI at 1 year. Pre- and post-contrast sagittal T1-weighted images show mild reduction in size of the gland (open arrowheads, c, d) and thinning of the pituitary stalk (open arrows, c,d).

Follow-up and Long-Term Outcomes

Clinical, radiological, biochemical and endocrine studies are needed during follow-up of patients with CDI without an identifiable cause. Pituitary stalk size at presentation is variable and can change with time (2429). In two large pediatric series of idiopathic CDI patients, PST was found in ~50–60% [30, 69]. Spontaneous evolution of thick pituitary stalk was similar in both reports from unchanged (30%) to reduction (30–50%) or further enlargement (10–20%). When the pituitary stalk is thickened, germinoma or LCH should be excluded, although other rare conditions such as neurosarcoidosis or granulomatosis with polyangiitis might be the underlying cause [118, 119].

Attention should be paid to LCH since long-term outcomes of bone or lung involvement are bad and highly suggestive of primary pituitary stalk-related LCH leading to CDI [30]. This raises a question about a possible relationship between idiopathic CDI and a single central nervous system target of LCH. Several self-limited cases of isolated CDI as the first sign of LCH developing shortly after the onset of polyuria and polydipsia may escape diagnosis emphasizing that early recognition of signs and symptoms of organ involvement by LCH should be carefully monitored in order to avoid a delayed diagnosis and consequent morbidity and mortality [30, 66, 120, 121].

MRI follow-up is recommended for all patients with an enlarged pituitary stalk every 6 months for the first 2 years after the diagnosis of CDI. In cases with a high likelihood of germinoma, MRI can be personalized (3 months) and pituitary stalk biopsy is advisable if the MRI reveals enlargement of either the pituitary stalk lesion (>6.5 mm) or the AP gland, or third ventricle involvement [25, 58, 69, 74, 122–124], with or without the presence of tumour markers.

Among patients with idiopathic CDI and a thick pituitary stalk, 90–94% developed AP hormone deficits with isolated GHD accounting for 60% of cases. Multiple pituitary hormone deficits were present in 30–50% of patients while only 10% of 19 patients with a normal pituitary stalk had an additional hormonal deficit [25].

39–80% of patients with CDI of different aetiologies have been reported with AP defects [25, 30, 31, 69, 122].

A systematic diagnostic approach and an appropriately tailored long-term follow-up enabled a precise diagnosis in 75 of 78 patients (96%) [30]. Twenty-four patients (28.2%) received an aetiological diagnosis at the time of presentation; 8 had LCH affecting the bone and/or skin, 6 craniopharyngiomas, 3 midline defects, 3 familial autosomal dominant CDI and 2 germinomas with double localization (pineal gland and pituitary stalk simultaneously) and the remaining 2 had post-traumatic CDI. Among the 61 patients (71.8 %) with a presumed idiopathic form of CDI, 7 (8.2%) were lost to follow-up within 2 years and 11 (13.0%) received a specific diagnosis within 2.5 years after presentation of CDI. 7 had germinoma and 4 of 11 were diagnosed with LCH. The remaining 43 patients (50.2%) were considered to have idiopathic CDI and underwent long-term clinical, endocrine and imaging studies; the median age at diagnosis was 7.4 years and they were followed for a median of 10.1 years. Initial stratification of the patients based on pituitary stalk size at the time of diagnosis showed 9 patients (20.9%) with a normal pituitary stalk, 27 (62.8%) with a minimal pituitary stalk thickness and 7 (16.3%) with moderate pituitary stalk thickness at the time of first MRI.

Serial MRI evaluations were performed over 5 years and at the time of adult height achievement. Of the nine patients with a normal pituitary stalk, six had minimal enlargement of pituitary stalk size during follow-up and this finding normalized in all patients within the second year and remained unchanged until reassessment at adult height achievement. Of the 27 patients with minimal pituitary stalk thickness, 15 had further enlargement at the second MRI. Five still had minimal PST at 5 years' evaluation (18.5%) but all had normal pituitary stalk at reassessment. Among the seven patients with moderate pituitary stalk thickness, four showed enlargement of pituitary stalk within 2 years after the diagnosis of CDI. Although reduction of pituitary stalk size was subsequently observed, none of these patients ever had a normal pituitary stalk. Thus 40 patients of 43 (93%) showed some pituitary stalk involvement within 6 months after the diagnosis of idiopathic CDI.

The results of AP function tests performed during the study period, and stratified by pituitary stalk thickness at diagnosis, showed that 35 patients (81.4%) developed at least 1 AP defect within the first 2 years and that a clear association was found between pituitary stalk thickness at diagnosis and the probability of developing pituitary defects. Recovery of endocrine function was documented in 46.5% of the patients suggesting that rescue of pituitary function is possible over time and that reassessment of pituitary function is mandatory.

Central Diabetes Insipidus and Adipsia

Although little is known about how the brain orchestrates systemic osmoregulation, advances have been made in understanding the molecular, cellular and network mechanisms that mediate the central control of homeostasis [10]. Despite the fact that cerebral osmoreceptors have a determining role in the control of osmoregulatory responses, peripheral osmoreceptors also contribute to fluid balance and patients with complex midline CNS abnormalities, such as septo-optic dysplasia, mainly have impaired AVP osmoregulation rather than frank CDI [38], which suggests that the disruption of osmoreceptor mechanisms contributes significantly to the aetiology of this disorder of homeostasis.

Adipsic disorders are characterized by inappropriate lack of thirst with failure to drink to correct hyperosmolality; adipsic patients with CDI have abnormally low thirst scores and no thirst response to marked plasma hypertonicity during hypertonic saline infusion [38]. Post-operative CDI and abnormalities of thirst occur in ~20% of patients with craniopharyngioma [125, 126], but only 2 of 159 patients with suprasellar tumours displayed adipsia [127], suggesting that hypernatraemia is seldom due to the tumour alone and that aggressive surgery is the main cause of adipsia.

Patients with craniopharyngioma who develop an adipsic syndrome and post-operative CDI fail to increase serum AVP in response to drug-induced hypotension and they do not feel thirsty after either a fall in blood pressure or hypertonic saline infusion, indicating that both osmotic and non-osmotic pathways are involved [77]. Failure to secrete AVP in response to hypotension or hypovolaemia may increase the risk of dehydration and life-threatening hypernatraemia that is associated with high mortality in hospitalized patients [128]. The mortality rate increases further both in the presence of infection [129] and unrecognized underlying secondary adrenal insufficiency. Adipsic CDI patients also manifest thromboembolism [130], behavioural disturbances, lethargy, coma and muscular weakness due to rhabdomyolysis [131]; some patients die of respiratory failure at relatively young ages. In a large cohort of 149 patients with CDI, 23 patients with concomitant adipsia had a higher risk of morbidity and mortality than the non-adipsic CDI patients [129].

Primary Polydipsia

Excessive water drinking suppresses vasopressin secretion [93] so the ingested water causes an increase in body fluids and a modest dilution of serum osmolality. The causes of primary polydipsia include psychogenic and

dipsogenic primary polydipsia but frank psychiatric compulsive drinking is an uncommon cause in children. Polyuria and polydipsia are reversible when the underlying cause improves. Patients are usually normonatraemic despite a large fluid intake but plasma sodium and osmolality may be low normal or slightly reduced.

Nephrogenic Diabetes Insipidus

NDI may occur with an intrinsic renal defect or may be acquired secondary to hypercalcaemia and hypokalaemia that impair the action of vasopressin on the distal nephron. Hereditary forms of NDI include X-linked NDI due to abnormalities of the gene for the vasopressin V2 receptor (AVPR2) in the kidney with resistance of renal tubules to the action of vasopressin and autosomal recessive or autosomal dominant NDI due to abnormalities of the AQP2 water channel [9, 29, 92]. A number of genetic mutations or deletions of the gene that encodes the vasopressin V2 receptor located on Xq28 have been identified. The V2 receptor is a seven-domain transmembrane protein, and genetic abnormalities have been located in the transmembrane domain as well as in the external and internal segments of the receptor. More than 180 different inactivating mutations in *AVPR2* have been described, all of which cause congenital NDI as a result of receptor malfunction at different levels, such as reduced receptor expression at the cell surface or disturbances in hormone binding and G-protein coupling. The autosomal recessive form accounts for 10% of patients with familial NDI.

The Syndrome of Inappropriate Antidiuretic Hormone Secretion

Causes of hyponatraemia in children include the syndrome of appropriate antidiuretic hormone secretion (SAADH) and the syndrome of inappropriate antidiuretic hormone secretion (SIADH). Although often less discussed and considered than SIADH, SAADH is by far the commonest cause of hyponatraemia in the pediatric population [132]. Cerebral salt-wasting syndrome (CSWS) as a cause of hyponatraemia may exist but is often diagnosed and reported without substantive evidence. Brain injury may result in the direct secretion of natriuretic factors that result in renal salt loss, volume concentration and AVP secretion, although the heart is the major source of most circulating NP. The clinical state, assessment of extracellular fluid volume, measurement of urinary electrolytes and responses to infusion of saline can distinguish these conditions.

Classical SIADH is diagnosed when other causes for hyponatraemia have been excluded. These comprise an absence of hypovolaemia with no evidence of diseases accompanied by oedema; no endocrine dysfunction, including adrenal insufficiency and hypothyroidism; no renal failure and no administration of drugs that can influence water homeostasis [133].

A novel disorder of water balance with an SIADH-like clinical picture has been termed 'nephrogenic syndrome of inappropriate antidiuresis' [134]. Two unrelated male infants with euvolaemic hyponatraemia and serum hypo-osmolality with inappropriately elevated urine osmolality and urinary sodium concentrations have been described. At first glance, the disorder in these boys resembles SIADH that is characterized by insufficient suppression of AVP secretion in relation to the degree of hypo-osmolality but serum AVP concentrations were undetectably low in these boys. It was postulated that a constitutively active V2 receptor could be the cause of the disorder and sequencing of *AVPR2* revealed a hemizygous point mutation. In an *in vitro* functional assay, the mutations were shown to lead to the production of a constitutively active V2 receptor. The consequence was AVP independent and therefore inappropriate activation of V2 receptor-mediated renal urine concentration. Remarkably, and for reasons that remain unexplained, neither patient showed clinical or biochemical signs of constitutive activation of extrarenal V2 receptors, which are known to mediate increases in circulating coagulation and fibrinolytic factors and a decrease in diastolic blood pressure after AVP stimulation.

Management of Central Diabetes Insipidus

The drug of choice for the treatment of CDI is desmopressin (dDAVP), a synthetic analogue of arginine vasopressin with a 2–3000-fold lower vasopressor effect. Desmopressin may be administered orally, intranasally or parenterally and each mode of administration has advantages and disadvantages. Desmopressin tablets appear to be safer and less frequently associated with hyponatraemia than the intranasal formulation [135, 136].

Maximum plasma concentrations are reached in 40–55 minutes after the administration of an oral or intranasal dose. The drug's half-life is 3.5 hours. Generally, urine output will decrease 1 or 2 hours after administration and the duration of action will range from 6 to 24 hours [137]. There is broad individual variation in the dose required to control diuresis. A low dose should be used initially and increased as necessary. Daily oral doses (20 times less potent than the intranasal form) vary from 100 to 1200 µg in three divided doses; intranasal doses are 2–40 µg once or twice a day; parenteral doses are 0.1–1 µg.

In early infancy, fluid management alone may be appropriate. When infants are treated with DDAVP, low doses of diluted oral preparations are usual. It is safe to allow a short diuresis between doses [138]. DDAVP stability is reduced by dilution so these preparations should not be used for more than 1 week. Indeed it may be best to make up the solution with each dose by crushing a DDAVP tablet and dissolving it in a small amount of water and then administering the required amount. Desmopressin, low renal solute load formula and thiazide diuretics have all been used in infancy to treat diabetes insipidus [139].

In older children, the intranasal spray (2.5–10 µg once or twice daily) or oral preparations are the preparations of choice for CDI. Oral desmopressin is particularly helpful in childhood. Its positive characteristics include better absorption, fewer complications and good compliance among children and adolescents due to the easy route of administration. Oral lyophilized desmopressin has been recently introduced but, although it has a 60% higher bioavailability than the tablet, dose adjustment is limited compared with the tablet formulation [135, 140, 141].

Symptomatic dilutional hyponatraemia is a potential hazard if desmopressin is administered in excess over a long time period. Symptoms include headache, nausea, vomiting and seizures, which can lead to coma and death, but asymptomatic hyponatraemia may also occur. Particular attention is required in cases of multidrug therapy because of the risk of extrapontine myelinolysis [142]. Regular measurement of electrolytes is required after initial commencement of DDAVP.

Rare side effects with intranasal delivery of DDAVP include eye irritation, headache, dizziness, rhinitis or epistaxis, coughing, flushing, nausea, vomiting, abdominal pain, chest pain, palpitations and tachycardia [143]. DDAVP during pregnancy is safe for the mother and her fetus/child [144].

In the presence of adipsia or hypodipsia, the management of DI is difficult and is best managed initially by adjusting the DDAVP dosage and fluid intake in a hospital setting. Adipsic patients should maintain regular use of DDAVP with regular and periodic water ingestion. A fixed daily fluid intake appropriate for weight to maintain a 'personalized' value of serum sodium concentration at which the patient is known to be 'eunatraemic' and 'euvolaemic' should be established. Desmopressin is then administered at a dose and frequency to establish an appropriate urine output and neutral fluid balance, allowing for insensible losses. Regular weighing and measurement of serum sodium concentrations are mandatory. Intravenous or nasogastric hydration may be required when the patient is unable to drink.

Treatment of Nephrogenic Diabetes Insipidus

No specific treatment aimed at restoring the function of the mutant V2 receptor to treat X-linked NDI is available [145]. Volume contraction and thiazide diuretics, amiloride and indomethacin act indirectly by decreasing the amount of tubular fluid presented to the distal tubule [146, 147]. Successful treatment has been reported with a combination of amiloride (20 mg/1.73 m^2/day or 0.3 mg/kg/day orally 3 times a day) and/or hydrochlorothiazide (1–3 mg/kg/day 2–3 times per day orally) and/or indomethacin (1–3 mg/kg/day 2–3 times per day orally). This treatment is most effective in patients with mild to moderate forms of X-linked NDI with partial loss-of-function mutations. Patients with mild to moderate disease are rare and most patients are unresponsive to AVP or DDAVP. Chemical chaperones represent a class of substances leading to a transport rescue of misfolded membrane proteins specifically from post-ER compartments, leading to a functional rescue of NDI. Pharmacological chaperone-based therapy could potentially become a general treatment for severe forms of NDI [148].

Treatment of Inappropriate Antidiuretic Hormone Secretion

The key to effective management of hyponatraemia is establishing the type and its cause, so that the cause can be removed if possible and appropriate management be instigated [149]. It is important to clarify whether hyponatraemia has developed quickly (over a few days) and is acute or whether it has developed over days to weeks and is chronic. The rapidity of correction of the serum sodium concentration should be closely linked to the suspected time over which the hyponatraemia has developed. If the patient has mild symptoms of hyponatraemia (headache, lethargy, dizziness) or is asymptomatic and the hyponatraemia is mild (sodium concentration >125 mmol/L), a conservative approach is recommended. Discontinuation of all possible offending drugs is important.

In SIADH or oedema-producing states, a trial of water restriction to <1–1.25 L/day (depending on the degree of hyponatraemia and the age) can be attempted; the serum sodium concentration should be measured at regular intervals to look for improvement. If the serum sodium level continues to fall, the patient may require an intravenous trial of normal saline to clarify the diagnosis. If the patient has ECF volume contraction as in CSWS (which may not be clinically apparent), a trial of saline will improve the serum sodium level [150], while in SIADH, the hyponatraemia will worsen. The trial should be performed with caution using 3–5% saline infusion at 0.1 mL/kg of

body weight/minute (up to 1–2 mL/kg of body weight/h). Rapid correction can result in osmotic demyelination syndrome leading to severe brain injury and death. Oral urea for the treatment of chronic syndrome of inappropriate antidiuresis in children has been reported to be successful; 30% oral urea solution at a starting dose of 0.1 g/kg/day divided into four doses and increased gradually to 2 g/kg/day [142].

In December 2005, conivaptan was approved by the FDA for the treatment of euvolaemic hyponatraemia, and in February 2007 this indication was extended by the FDA to include hypervolaemic hyponatraemia [151]. Oral tolvaptan therapy in adult patients with the SIADH secretion has been reported [152] and a clinical trial in children has been started recently.

Challenges

At the present time, the management of diabetes insipidus remains a challenge in many cases. Early diagnosis of the underlying cause of CDI is advisable, and future research is needed in order to identify the role of specific antigens and autoantibodies for autoimmune CDI-related conditions. In addition the identification of new early serum and CSF markers of germ cell tumours and LCH is desirable. Further experience in imaging techniques is required to better define the diagnostic yield of whole body STIR imaging, and 18F-FDG PET and amino acid PET tracers deserve further investigation to assess the potentially promising role of this imaging modality. Future developments in whole body DWI and hybrid PET/MRI systems are expected to reveal additional insights.

Conclusions

Water homeostasis is critical to all mammals and maintenance of the tonicity of extracellular fluids is crucial for cell function. The maintenance of water balance in healthy humans is achieved by thirst, vasopressin and kidney function. Renin–angiotensin–aldosterone and NP also regulate water and electrolyte balance. Vasopressin and its protein carrier NPII are released from the posterior pituitary when the axon is depolarized by osmoreceptor or baroreceptor stimuli. AVP activates the insertion of aquaporin 2 – a water channel – vesicles into the cell membrane of the collecting duct. In diabetes insipidus, large volumes of dilute urine (polyuria) are excreted due to vasopressin deficiency (CDI), vasopressin resistance (nephrogenic diabetes insipidus) or excessive water intake (primary polydipsia). Primary polydipsia – both psychogenic and dipsogenic – is characterized by excessive water drinking that suppresses vasopressin secretion. Frank psychiatric compulsive drinking is uncommon in children. Patients usually remain normonatraemic despite large fluid intake.

Polyuria must be evaluated by means of a water deprivation test, with measurement of plasma and urine osmolality, and a desmopressin challenge should be performed if diabetes insipidus has been diagnosed. CDI may be due to the destruction of neurons originating in the supraoptic and PVN because of intracranial germ cell tumours, LCH, inflammatory/autoimmune conditions, vascular diseases, surgical or accidental trauma, metastases, midline cerebral and cranial malformations and other rare conditions such as tuberculosis and neurosarcoidosis. In other cases, genetic defects in AVP synthesis or other genetic diseases are the underlying cause. Patients with CDI and adipsia have a significantly higher risk of morbidity and mortality than in non-adipsic diabetes insipidus. NDI is secondary to AVP receptor-2 mutations, abnormalities of aquaporin 2 water channel or other intrinsic kidney disorders.

History, patient's age at disease onset and clinical examination may provide important clues to possible underlying diagnoses. MRI with current high field systems is the modality of choice for evaluating the hypothalamic–pituitary axis in CDI. Pituitary stalk size evolution should be monitored. Additional important diagnostic information can be obtained by clinical, radiological, biochemical and endocrine follow-up studies. Patients with idiopathic CDI and a thickened pituitary stalk are likely to develop AP hormone deficits.

The drug of choice for the treatment of CDI is oral, intranasal or parenteral desmopressin. Dilutional hyponatraemia is a potential hazard if desmopressin is administered in excess over a prolonged time period. NDI can be treated by volume contraction and thiazide diuretics, amiloride and indomethacin.

The SIADH secretion and CSWS are causes of hyponatraemia. Clinical evaluation, assessment of extracellular fluid space volume status, measurement of urinary electrolytes and responses to infusion of saline solutions can distinguish these conditions; therapy (water restriction/oral urea/vaptans or saline infusion) must be carefully chosen after determining the cause of hyponatraemia.

Clinical Guidelines

- Polyuria is characterized by urine volume in excess of 2 L/m^2/24 h or ~150 mL/kg/24 h at birth, 100–110 mL/kg/24 h until 2 years and 40–50 mL/kg/24 h in the older child and adult.

Accurate 24 hours' evaluation of water intake and urine output is mandatory and the presence of polyuria in the absence of solute diuresis (glucose, calcium) should rise the suspicion of diabetes insipidus. In infants or young children, random morning plasma osmolality after discontinuing water intake at midnight (>295 mOsm/kg H_2O) and/or serum sodium (>143–145 mEq/L) associated with urine hypo-osmolality (<300 mosm/L or urine/plasma osmolality ratio <1) are diagnostic of complete or partial (urinary osmolality 300–500 mosm/L) diabetes insipidus. When the osmometer is not available, a good estimation of plasma osmolality can be obtained with the following formula where 18 and 2.8 represent the molecular weights of glucose and blood urea nitrogen (BUN), respectively, if originally expressed in mg/dL:

$$\mathrm{POsm} = 2\left[\mathrm{Na}+\right] + \frac{\mathrm{glucose}\left(\mathrm{mg/dlL}\right)}{18} + \frac{\mathrm{BUN}\left(\mathrm{mg/dlL}\right)}{2.8}.$$

In the absence of a straightforward diagnosis, water deprivation test and DDAVP trial are needed. A 7 hours (or less) deprivation test is usually sufficient for diagnosis, except in cases of primary polydipsia, where a longer dehydration period is sometimes required. The test must be discontinued if weight loss exceeds 5% of starting weight and/or plasma Na+ is found to be higher than 143–145 mEq/L and/or plasma osmolality is higher than 295–300 mOsm/kg H_2O and/or urine osmolality increases to normal.

The administration of 5–10 μg of desmopressin (1-deamino-8-D-arginine vasopressin, DDAVP) intranasally will help to make a differential diagnosis between central and nephrogenic diabetes insipidus (CDI and NDI).

- In diabetes insipidus, history, patient's age at disease onset and clinical examination may provide important clues to possible underlying diagnoses.
- Magnetic resonance imaging with current high field systems represents the modality of choice for evaluating the hypothalamic–pituitary axis in CDI. Pituitary stalk size evolution should be monitored. Additional important diagnostic information can be obtained by clinical, radiological, biochemical and endocrine follow-up studies. Serum and cerebrospinal fluid germ cell tumour markers – human chorionic gonadotropin, placental alkaline phosphatase and alpha-fetoprotein – may be detectable in germ cell tumours. Careful attention to other signs over time, such as recurrent otitis media, skin lesions, cholangitis, dyspnoea or bone pain/lesions, is mandatory in order to rule out multi-organ involvement by Langerhans cell histiocytosis. The diagnostic role of antibodies against vasopressin-secreting cells (AVPc-Abs) and pituitary antibodies remains questionable.
- Patients with idiopathic CDI and thickened pituitary stalk are likely to develop anterior pituitary hormone deficits; monitoring of anterior pituitary function is therefore mandatory in CDI patients.
- The drug of choice for the treatment of CDI is oral, intranasal or parenteral desmopressin (dDAVP), a synthetic analogue of the endogenous hormone arginine vasopressin, but with a lower vasopressor effect. Dilutional hyponatraemia is a potential hazard if desmopressin is administered in excess over a prolonged time period.
- Volume contraction and thiazide diuretics, amiloride and indomethacin are indirect forms of treatment for NDI.
- The syndrome of inappropriate antidiuretic hormone secretion (SIADH) and cerebral salt-wasting syndrome (CSWS) are causes of hyponatraemia. Clinical evaluation, assessment of extracellular fluid space volume status, measurement of urinary electrolytes and responses to infusion of saline solutions can distinguish these conditions; therapy (water restriction/oral urea/vaptans or saline infusion) must be carefully chosen after determining the cause of hyponatraemia.

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15

Diabetes Mellitus

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KEY LEARNING POINTS

- Although there are many forms of diabetes in childhood and adolescence, type 1 diabetes remains the commonest form. Hence diabetes should be initially treated with insulin unless there is a compelling reason to do otherwise.
- The significant acute complications of type 1 diabetes (diabetic ketoacidosis, hypoglycaemia) still occur commonly despite numerous changes in therapy. The association, however, between a lower HbA1c and increased rates of severe hypoglycaemia appears to be no longer present.
- There are a variety of insulin regimens that are all effective in the treatment of type 1 diabetes.
- Good management of type 1 diabetes and achievement of target markers of metabolic control will significantly lower the risk of microvascular and macrovascular disease and promote longevity.
- Type 2 diabetes in children and adolescents is largely limited to at risk ethnic groups and, despite the obesity epidemic in children, remains less common than other forms of diabetes in most ethnically mixed populations. Diabetes-related complications may already be present at the time of diagnosis.
- There are numerous forms of monogenic diabetes and although uncommon, these should be suspected in individuals who do not have markers of autoimmunity and have a family history or early-onset diabetes.
- Areas of research to improve clinical care are focused around mechanical/technologic solutions such as closed-loop insulin delivery systems and biologic solutions such as regenerating beta cells or designing glucose sensing/insulin delivering cells that are not damaged by the immune system.

Type 1 Diabetes

Epidemiology

The incidence of diabetes varies strikingly by geographical regions and is rising by 2–4% each year. The latest International Diabetes Federation (IDF) estimates show that 542,000 children worldwide are affected with type 1 diabetes mellitus (T1DM), with 86,000 new cases diagnosed per year, reflecting an annual increase in the incidence of 3% [1]. Large registries and multicentric studies (e.g. DIaMonD, EURODIAB and the SEARCH studies) have provided most of the epidemiological data but there is bias associated with such registries because countries with poor health infrastructure and surveillance systems are underrepresented.

The Multinational Project for Childhood Diabetes (DIaMonD) was set up in 1990 by the World Health Organization to monitor the incidence patterns of T1DM [2] per 100,000/year in children aged ≤14 years from 114 populations in 112 centres in 57 countries. Striking differences by region were found, with a 400-fold difference between geographic regions; China and Venezuela had incidence rates of only 0.1 per 100,000/year compared with 40.9 per 100,000/year in Finland. Scandinavia and the UK had the highest rates of diabetes in Europe with intermediate rates in central Europe and lowest rates in southern Europe (Figure 15.1). A north–south gradient

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in incidence has been proposed, the chief exception being Sardinia, which has the second highest rate in the world. Most Asian populations have low incidence rates with the exception of Kuwait, which had an incidence of 22 per 100,000/year. In Oceania, the incidence ranged from 14 per 100,000/year to 22 per 100,000/year. The incidence in South American populations varied between very low to high. Data from Africa are scarce but seem to show a low incidence but estimates may not be accurate because of high childhood mortality rates.

The SEARCH for Diabetes in Youth (SEARCH) study was designed to assess the prevalence and incidence of diabetes in young persons <20 years of age overall and by type, age, sex and ethnicity [3]. In 2001, 4958 diagnoses with T1DM in a population of 3.3 million gave a prevalence of 1.48 per 1000; in 2009, the prevalence was 1.93 per 1000 (6666 cases of 3.4 million). Striking ethnic differences in prevalence rates were found, the highest 2009 rates being 2.55 per 1000 among white young persons and the lowest being 0.35 per 1000 in American Indians.

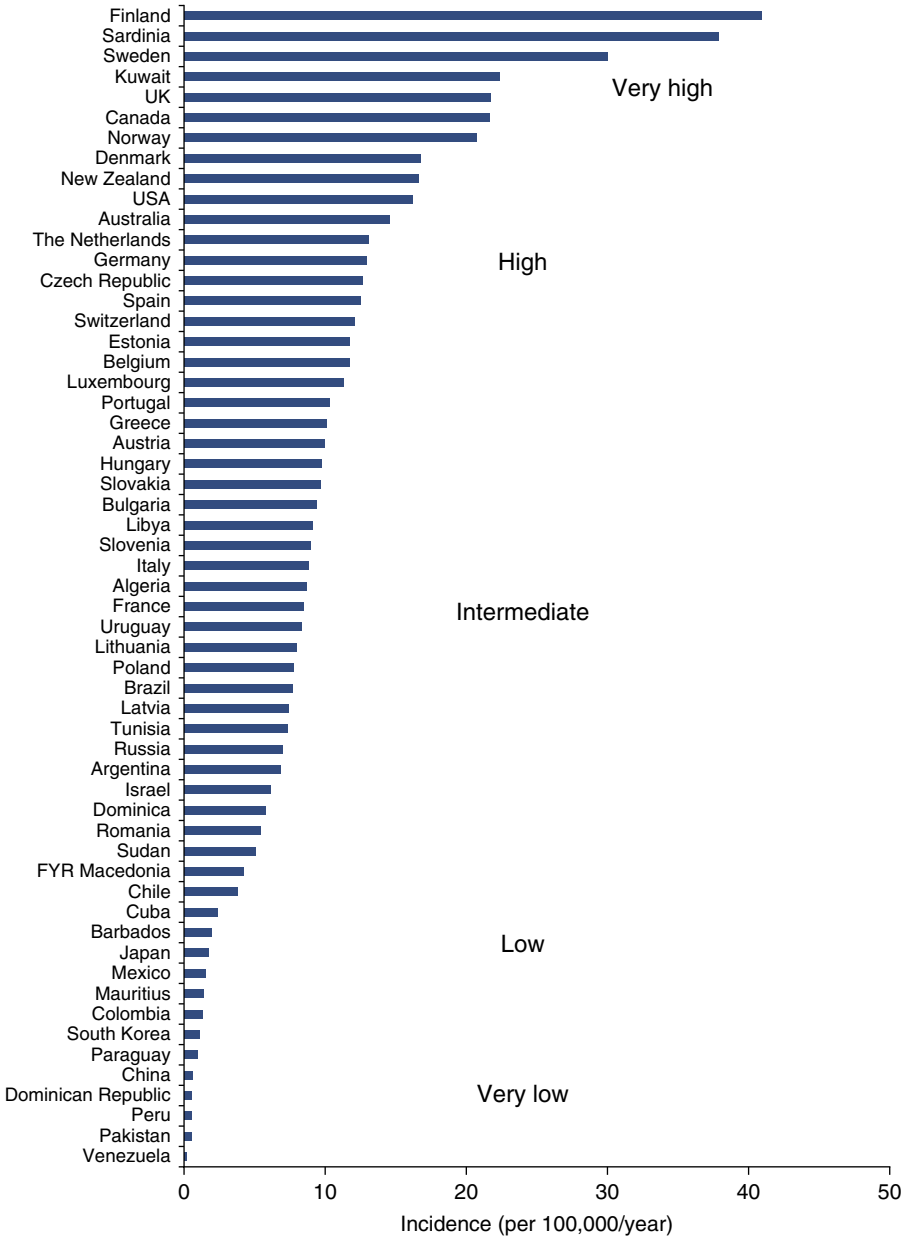

Figure 15.1 Age-standardized incidence of T1DM in children under 14 years of age (per 100,000 per year). Countries are arranged in descending order according to the incidence [2]. *Source:* Reproduced with permission of Springer.

No gender-specific differences in incidence have been described for childhood T1DM but there is a male preponderance reported in adults, which is contrary to most other autoimmune disorders [4]. The incidence appears to peak around puberty.

Trends in Incidence with Time

The incidence of childhood-onset T1DM has been increasing worldwide, with the WHO DIaMonD project yielding estimates of the overall annual increase of 2.4% in 1990–1994 and 3.4% in 1995–1999 [2]. Data from the EURODIAB study group showed 20 year incidence rates in 1989–2008 from 23 centers in 19 countries registering 49,969 new cases of T1DM in individuals diagnosed before their 15th birthday. The study showed stable incidence rates of 3.4% per annum and 3.3% per annum in the first and second decades of reporting. This increase is not uniform and countries such as Sweden and Finland, which had showed a rapid rise in T1DM incidence during the past two decades, have shown plateauing of their rates recently. The most striking increase in incidence rates have been seen in Central and Eastern Europe indicating the trend that the countries with the lowest incidence rates generally showed the highest increases. There was an alarming trend towards increased incidence rates in under 5 year olds [5, 6].

The reasons for these changes are not clear but the rapidity of the changes suggest a significant contribution of environmental factors [7]. Genetic changes are unlikely to be responsible because the rising incidence of T1DM in childhood occurred in cases with lower-risk HLA genotypes [8].

The increase in the number of cases in the younger population has important epidemiological implications. Younger children have more acute presentations, with diabetic ketoacidosis leading to increased hospital admissions and risk of mortality [9, 10]. The lowering of age of development of T1DM means that these children will also be exposed to the diabetic milieu for longer, increasing their risk for developing long term micro- and macrovascular complications. All of these factors will affect healthcare costs.

Pathophysiology

T1DM is a chronic autoimmune disorder precipitated by environmental factors in genetically susceptible individuals (Figure 15.2). Several silent events precede clinically

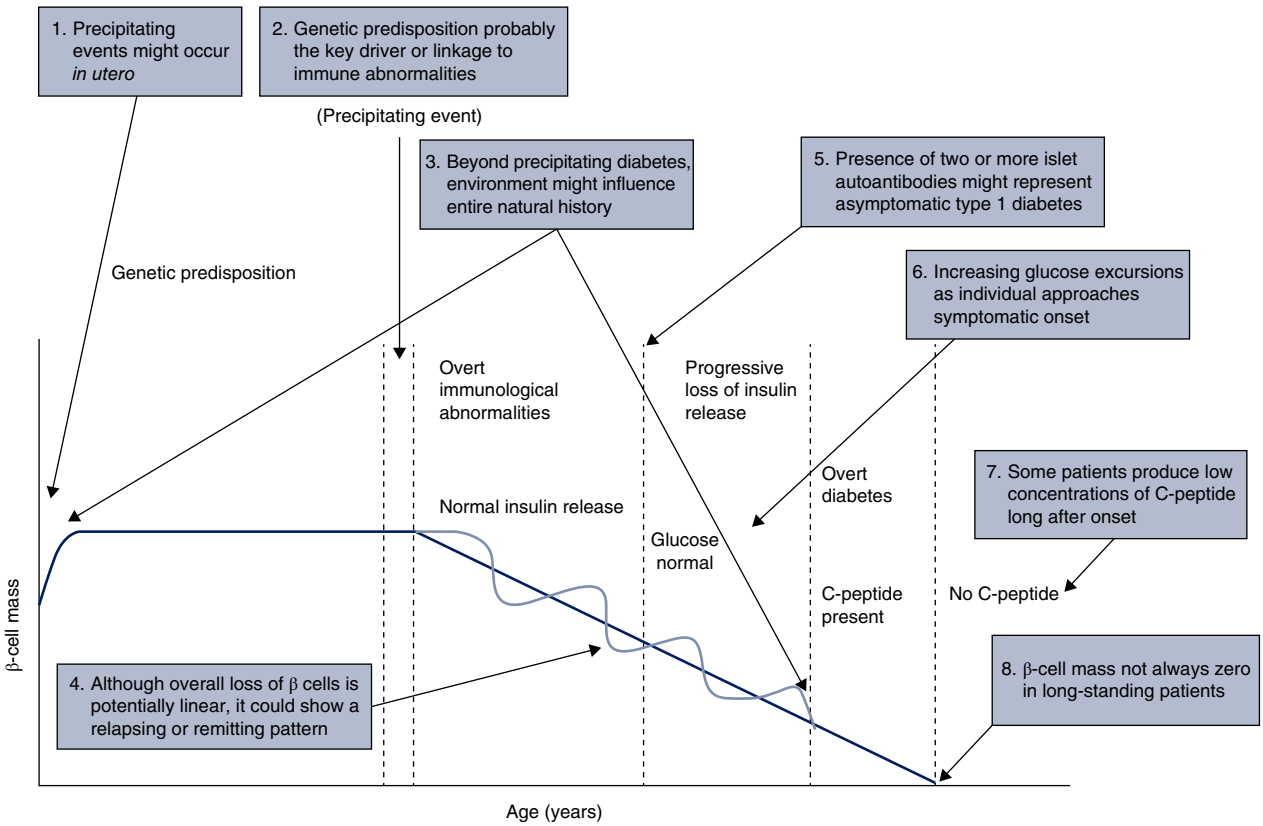

Figure 15.2 The modified 'Eisenbarth' model of the natural history of T1DM [11]. *Source:* Reproduced with permission of Elsevier.

evident diabetes. There is formation of reactive autoantibodies followed by the infiltration of self-reactive T cells in the pancreas leading to β-cell apoptosis [12].

Genetic Studies

T1DM has a polygenic inheritance. The development of disease is determined by an interaction between genetic predisposition and environmental triggers. Although more than 85% of cases are sporadic, family members of patients have an increased risk of developing diabetes compared to the general population [13]. Siblings of patients have a 6% lifetime risk of developing diabetes when compared with the general population risk of 0.4%. The risk also varies with the gender of the parent affected, with offspring of mothers with T1DM having a 3–4% risk whereas the risk maybe up to 6–9% with an affected father [14].

The two primary approaches that have been used to study susceptibility genes for T1DM have been linkage studies (using pairs of affected relatives, typically siblings) and association studies (using either case–control or family-based designs). Linkage studies are used when the genes being sought are rare but have a large effect. Association studies are used for genes that are more common in the population but have a smaller effect [15]. Most of the initial studies of T1DM susceptibility genes focused on individual candidate gene studies. Several defined loci have been identified (Table 15.1) but, with the advent of genome-wide association studies, more than 40 other susceptibility loci have been identified. The effect of these genes is at best modest with Odds ratios of <1.3. Most of the loci are associated with immune regulation [16].

Human Lymphocyte Antigen (HLA) Genes

The strongest evidence of linkage for T1DM has been in the HLA region on chromosome 6p21, which explains more than 50% of the genetic susceptibility. No other single genetic region confers a comparable risk. At least one of the two HLA class II haplotypes, DR4–DQ8 and DR3–DQ2, are present in ~90% of children with T1DM [14]. The highest risk is conferred by the DR3/DR4–DQ8 genotype followed by DR3 and DR4 homozygosity [17]. This high-risk genotype is seen in only 2.4% of the general Caucasian population as studied by newborn screening blood spots [18]. The DRB1*1501-DQA1*0102-DQB1*0602 haplotype, found in about 20% of the population but in only 1% of patients, confers dominant protection against T1DM [19].

Data from the Diabetes Autoimmunity Study in the Young (DAISY) has shown that risk for islet autoimmunity dramatically increased in DR3/DR4–DQ8 siblings who shared both HLA haplotypes with their diabetic proband sibling (63% by age 7; 85% by age 15) compared with siblings who did not share both HLA haplotypes with their diabetic proband sibling (20% by age 15). 55% of those children who shared both HLA haplotypes with their diabetic sibling developed T1DM by age 12 versus 5% of those sharing zero or one haplotype [20].

Longitudinal data from the Type 1 Diabetes Genetics Consortium (T1DMGC) have shown a decrease of the highest-risk HLA–DR3/DR4–DQB1*0302 genotype over time. There was an increased percentage over time of other HLA genotypes without HLA–DR3 or –DR4 in T1DMGC new onsets [21]. This trend could possibly indicate an increase of disease penetrance in individuals with lower-risk HLA genotypes and/or a lower disease penetrance in subjects with high-risk genotypes. A consistent association with MHC class 1 alleles, which can act in addition to and also independently of class 2 alleles has been shown. The MHC class 1 products play an important role in antigen processing and presentation to CD8+ cytotoxic T cells and may thereby have a plausible role to play in T1DM risk predisposition. The most significantly type 1 diabetes-associated alleles have been found to be B*5701 (protective) and B*3906 and HLA-A*02 (susceptible) [22].

Insulin (INS) Gene

An association has been described between T1DM and the insulin (*INS*) gene region on chromosome 11p15.5 (insulin-dependent diabetes mellitus 2, IDDM2). IDDM2 consists of a highly polymorphic stretch of 14–15 bp repeats of DNA lying 365 bp upstream of the transcriptional initiation site of the *INS* gene. There are three classes of variable number of tandem repeats (VNTRs) in the insulin gene: class I (26–63 repeats), class II (~80 repeats) and class III (140–200 repeats) [23]. Allelic variation at INS-VNTR confers differential susceptibility to T1DM. Homozygosity for class I VNTR determines high risk for T1DM, while class III VNTR confers dominant protection. It is hypothesized that allelic variation in the size of repeats modifies the insulin expression levels in the thymus by affecting AIRE binding to its promoter region. Consequently, VNTR type I induces lower transcription of insulin and its precursors in the thymus, leading to reduced tolerance and T1DM development [24].

Table 15.1 Defined susceptibility loci for T1DM.

Locus	Chromosomal location	Gene and function
IDDM1	6p21·31	HLA DR/DQ region
IDDM2	11p15·5	Insulin-VNTR
IDDM12	2q33 1p13	CTLA4 PTPN22

Protein Tyrosine Phosphatase, Non-Receptor Type 22 (PTPN22) Gene

The lymphoid-specific phosphatase LYP, encoded by the *PTPN22* gene on chromosome 1p13, is involved in preventing spontaneous T-cell activation. A nonsynonymous single nucleotide polymorphism (SNP) at nucleotide 1858 in codon 620 of this gene results in amino acid substitution (Arg620Trp). It is hypothesized that 620Trp decreases the affinity of LYP to the negative regulatory kinase Csk. The C1858T SNP in the *PTPN22* gene has been found to be associated with several autoimmune disorders, including T1DM in Caucasians [25, 26].

Cytotoxic T-Lymphocyte-Associated Protein 4 (CTLA4) Gene

The *CTLA4* gene is expressed only on activated T lymphocytes and downregulates T-cell function. By inhibiting further proliferation of activated T cells, limiting both activation and expansion [27]. Mutations or polymorphisms that lead to reduced activity of CTLA4 are believed to play an important role in autoimmunity [27]. Knockout mice models have been shown to develop lethal lymphoproliferative disorders [28]. Polymorphisms in the *CTLA4* gene have also been linked to other autoimmune disorders such as Graves and Addison diseases [29, 30].

Other Genetic Loci

Other loci that have been independently associated with T1DM susceptibility are IL2RA and IFIH1, both of which are linked to immune responsiveness [31].

Serological Studies

Autoantibodies are markers of T1DM and can be detected long before the onset of clinical disease (Figure 15.3). The main autoantibodies are islet cell antibodies (ICA), insulinoma-associated antigen-2 (I-A2, ICA512), anti-insulin (IAA), glutamic acid

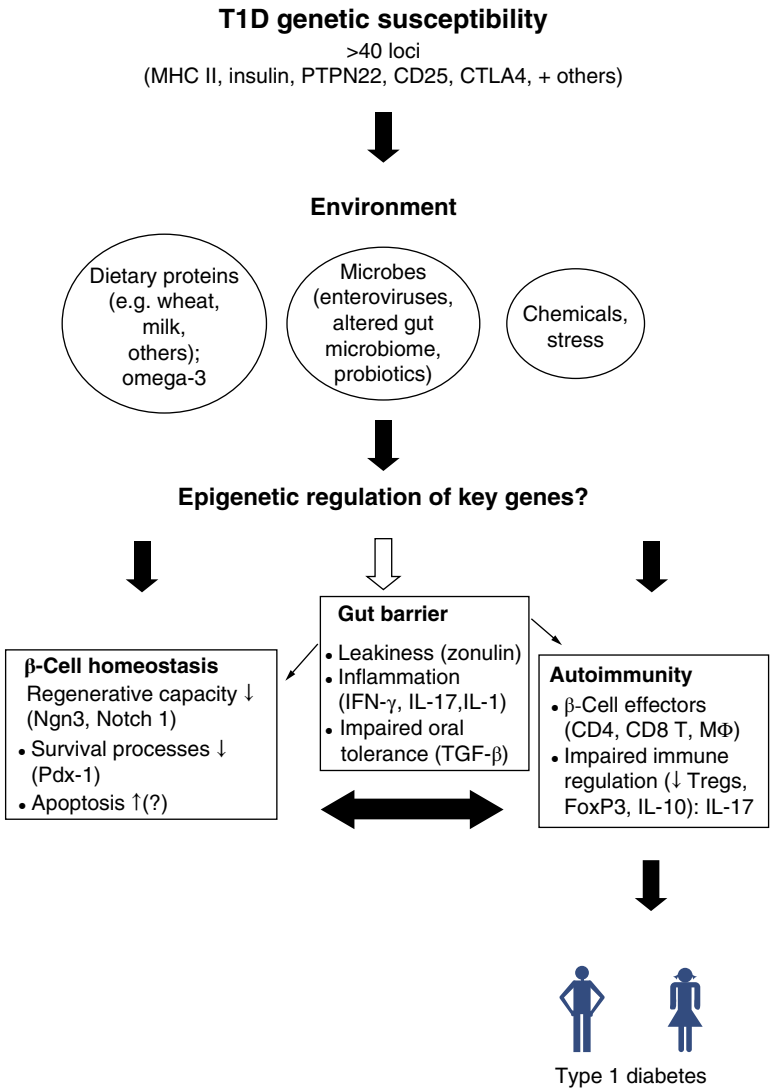

Figure 15.3 Possible role of epigenetic factors in environmental modification of T1DM risk [32].
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Table 15.2 Sensitivity of the major islet autoantibodies for the diagnosis of new-onset T1DM.

Autoantibody	Sensitivity (%)
ICA	70–80
GADA	70–80
IA-2A	60
IAA	60–80
ZnT8	60

decarboxylase 65 (GAD65) and zinc transporter 8 (ZnT8) (Table 15.2) [31, 33]. More than 90% of newly diagnosed T1DM cases are positive to at least one of these, which are used in disease prediction models [34, 35].

The first antibodies discovered were ICA detected by immunofluorescent techniques using human blood group O pancreas as substrate [36]. ICA is positive in about 70–80% of new-onset diabetic individuals but levels decline after diagnosis [37]. The assay is labour intensive and prone to variability in interpretation and so they are not used universally in diabetic autoantibody panels. Insulin is the only known β-cell-specific autoantigen and higher anti-IAA titres are found in children younger than 5 years of age. This pattern is also consistent with a faster rate of disease progression in this age group [38, 39].

IAA are seen in 50–60% of young children with new-onset T1DM. Samples for IAA estimation should be collected before insulin administration is initiated. Insulin-treated patients frequently develop insulin antibodies to the exogenous insulin that cannot be distinguished from IAA by the IAA assay [37]. GAD antibodies, similar to ICA, are positive in 70–80% of subjects with new-onset T1DM; they remain positive for longer and are therefore useful for diagnosis of LADA in adults [40]. IA-2A is less common at the onset of T1DM (~60%) than either ICA or GAD [37]. ZnT8 has recently been described as a major T1DM autoantibody [41]. ZnT8 autoantibody positivity is seen in 60–80% of newly diagnosed subjects [33] with levels declining rapidly in the first few years after diagnosis [42]. ZnT8 positivity was seen in 26% of subjects classified as autoantibody negative on the basis of negativity to GAD, IA2, IAA and islet cytoplasmic autoantibodies. The combined measurement of ZnT8A, GADA, IA2A and IAA raised autoimmunity detection rates to 98% at disease onset.

Autoantibodies and Diabetes Risk Prediction

Many preventive trials, such as the European Nicotinamide Diabetes Intervention Trial (ENDIT) [43], Diabetes Prevention Trial–Type 1 (DPT-1) [44] and DAISY [38], have shown that the presence of multiple autoantibodies significantly increases the risk of progression to clinical diabetes within 10 years. A recent analysis of pooled data from The American DAISY, the Finnish Type 1 Diabetes Prediction and Prevention (DIPP) and the German BABYDIAB and BABYDIET studies showed that progression to T1DM in children with multiple autoantibodies was 70% after 10 years of seroconversion and 14.5% in children with only one autoantibody [45]. In children who had no autoantibodies the risk of progression was only 0.4%. Age of onset of autoantibodies was found to have a significant association with onset of disease with much faster progression in children who developed antibodies before 3 years of age [45].

Role of Autoantibodies and B Cells in Pathogenesis

Current thinking is that autoantibodies are markers of autoimmunity and not primarily pathogenic. Emerging studies in NOD mice suggest that B cells are an important component in the pathogenesis of T1DM by virtue of their ability to act as the preferential antigen-presenting cell population required for efficient expansion of diabetogenic CD4+T cells [46].

Other Serological Markers of Autoimmunity

Some metabolic markers can act as surrogate markers of autoimmunity. Specific metabolic derangements can precede the onset of islet autoantibodies and thus act as very early markers of disease. Individuals who developed diabetes had reduced serum levels of succinic acid and phosphatidylcholine (PC) at birth, reduced concentrations of triglycerides and antioxidant ether phospholipids throughout the follow-up and increased concentrations of pro-inflammatory lysoPCs several months before seroconversion to autoantibody positivity [47]. Autoantibody-positive children have been found also to have higher concentrations of odd-chain triglycerides and polyunsaturated fatty acid-containing phospholipids and lower concentrations of methionine than autoantibody-negative children [48].

Environmental Factors

Environmental factors are thought to play a significant role as triggers and potentiators of β-cell destruction. There are various reasons that support the role of an exogenous agent in inducing autoimmunity, which include <50% concordance rate in monozygotic twins, the significant proportion of susceptible HLA-carrying individuals who do not progress to clinical disease, the rapid rise in incidence across the world, the geographical variation in disease and migrant studies of low incidence populations that move to high incidence countries acquiring local disease risk rates [49]. The specific environmental factors, which may trigger T1DM, have been

the subject of intense investigation. Most of the research has focused on infections, the role of diet and toxins.

Viral Infections

Viruses have been considered as possible triggers that lead to onset of clinical diabetes in genetically predisposed individuals. Enteroviruses have most commonly been implicated. The potential mechanisms by which a virus may initiate autoimmunity are direct β-cell destruction, molecular mimicry between enteroviral proteins and GAD65 antigen, bystander activation and persistent infection and inflammation leading to recruitment of autoreactive T cells and loss of immune tolerance. The evidence supporting a link between infection and β-cell autoimmunity includes higher serum anti-enteroviral antibodies in diabetic individuals, detection of viral RNA in the pancreas of people with T1DM, detection of viral RNA in peripheral blood and persistent enteroviral infection of small intestinal mucosa in people affected with T1DM [49]. Data from the DIPP study have shown a temporal association between enteroviral infection and appearance of a first diabetic autoantibody [50] but data from the BABYDIAB study [51] and the DAISY study failed to show any association between enteroviral infection and the development of T1DM [52]. The Environmental Determinants of Diabetes in the Young (TEDDY) also found no evidence of a temporal relationship between viral infection and seroconversion in young children with rapid-onset T1DM [53].

While the debate around the potential role of viruses continues, there is evidence that the incidence of T1DM is higher in developed countries where the prevalence of enteroviral infections has significantly decreased with time. This paradox is explained by the hygiene hypothesis, which states that viral infections during childhood may protect individuals from the risk of developing T1DM or delay disease onset. Children from populations where the background risk of enteroviral infections is high may have built up a strong immune response to these agents, thereby making them less susceptible to the detrimental effects of infection [54, 55]. Despite associative data between viral infections and islet autoimmunity, no direct causal relationships have been demonstrated. Whether viral agents act just as modifiers/accelerators of an ongoing autoimmune process or actual triggers remains to be established.

Diet

Dietary factors, such as cow's milk protein, gluten exposure and vitamin D, have long been implicated in the pathogenesis of T1DM.

Duration of breastfeeding and/or early exposure to cow's milk protein has been proposed to precipitate autoimmunity in T1DM. Various constituents of cow's milk such as bovine insulin, bovine serum albumin and casein are candidate molecular factors that may act as triggers for islet autoimmunity [49]. The data supporting the role of cow's milk as a trigger have been contentious with studies showing higher risk, lower risk and no difference in risk [56]. Data from prospective cohort studies such as DAISY and the BABY-DIAB studies, however, did not find that early exposure to cow's milk nor reduced breastfeeding duration was associated with development of β-cell autoimmunity [57, 58].

The DAISY study has shown that HLA risk genotype may modify the effect of cow's milk and risk of T1DM: greater cow's milk protein intake was associated with increased islet autoimmunity risk in children with low/moderate risk HLA–DR genotypes but not in those with a high risk genotype [59]. The Trial to Reduce IDDM in the Genetically at Risk (TRIGR) study was designed to address the issue of cow's milk protein and risk of progression to T1DM definitively by assessing whether weaning to an extensively hydrolysed formula or a conventional cow's milk-based formula before the age of 6–8 months affected the development of islet autoimmunity. The pilot TRIGR study, which was carried out only in Finnish children, showed that those children who were weaned to the extensively hydrolysed formula had a 50% reduction in the cumulative incidence of β-cell autoimmunity by the age of 10 years [60]. A more recent publication from the TRIGR group reported results from the larger multicentric cohort, which failed to show a similar association: the use of an extensively hydrolysed formula did not reduce the incidence of diabetes-associated autoantibodies after 7 years [61]. The potential association between cow's milk protein and risk of islet autoimmunity remains controversial.

Plant proteins have been implicated as potential environmental triggers of islet autoimmunity. Data from the DAISY study have shown that both early (<4 months of age) and late (≥6 months of age) first exposure to solid foods and gluten influenced the risk of development of islet autoantibodies and progression to T1DM [62, 63]. Data from the BABY-DIAB study also showed that early exposure to gluten before the age of 3 months increased the risk of development of islet autoimmunity [58]. These studies implied that adhering to conventional infant feeding guidelines might lessen the risk of progression to clinical diabetes. The prospective German BABYDIET study explored the effects of delaying gluten exposure on risk of islet autoimmunity and found no difference in progression of development of islet autoantibodies in the late gluten exposure (>12 months) group compared with gluten exposure between 6 and 12 months of age [64].

Vitamin D has been linked to islet autoimmunity due its immunomodulatory properties. An early Finnish birth cohort study found that children who had been

supplemented with high dose vitamin D of 4000 units had a lower risk of T1DM development compared to those supplemented with lower doses [65]. More recent meta-analysis found that the risk of development of T1DM was lower in the supplemented infants compared to those who were not but these associations varied between studies. Most recent studies have failed to show reduction of risk with vitamin D supplementation or association between vitamin D concentrations and risk of disease onset [66, 67]. Vitamin D receptor polymorphisms have been shown to have some association with T1DM development [68] and links between vitamin D-binding protein concentrations and T1DM have been proposed [69].

There are some strong arguments against the role of vitamin D as a potential trigger for the autoimmune process in T1DM, the most significant being that the risk of T1DM is highest in developed countries where almost all children receive supplementation, at least in infancy. Any causal relationship between vitamin D and T1DM remains an open question [56].

Epigenetic Studies

Epigenetics refers to heritable changes in gene expression that do not involve changes to the underlying DNA sequence, i.e. a change in phenotype without a change in genotype. Epigenetic regulation is a process by which mammals respond to environmental exposures [32]. Epigenetic mechanisms include methylation of DNA, the activation of microRNAs or post-translational modification of histones [70]. Epigenetic modifications are a potential explanation for the heterogeneity in T1DM presentation and pathogenesis and increase in disease incidence (Figure 15.3). Epigenetic modification and change of gene expression profiles may also affect β-cell development, function and repair response. Immune responses such as T-cell maturation, cytokine gene expression and Treg responses also need appropriate epigenetic regulation. Hyperglycaemia has itself been shown to lead to histone modification [71].

DNA Methylation

Studies of epigenome-wide association in CD14+ monocytes from T1DM-discordant monozygotic twin pairs have identified T1DM-associated methylation variable positions (T1DM-MVPs) that are significantly correlated with the diabetic state and antedate clinical disease. The genes showing differential methylation were mapped to several known T1DM-associated genes: *HLA*, *GAD2*, *INS*, *IL-2RB* and *CD226* [72, 73].

Activation of microRNA (miRNA)

miRNA's are involved in post-translational regulation of gene expression. They can modulate gene function by translational repression and affect the control of cellular processes and disease pathogenesis. This property of miRNA may be necessary for normal β-cell development and function. miRNA's are released in the extracellular space after cell death and may act as biomarkers of β-cell destruction [70]. Circulating levels of miR-375, which is expressed at high levels in islet cells, have been found to be raised in NOD mice before the onset of hyperglycaemia and can be used as a marker of β-cell death and potential predictor of diabetes [74]. Human studies have found elevated levels of mir-326 and differential miRNA profiles in Treg cells in T1DM patients [75, 76].

Histone Modification

Histone modification can lead to alteration of the chromatin structure and thereby affect gene expression and repression [70]. Hyperglycaemia has been shown to affect histone methylation [71]. Histone acetylation status of HLA class II loci has been found to be markedly different in monocytes from patients with T1DM monocytes and this is thought to affect response to external stimuli and modify T1DM susceptibility risk [77]. *CTLA4*, another T1DM susceptibility gene, has also been found to have differential histone methylation [78].

Epigenetic Interventions and Future Markers

A recent trial has tested the use of an epigenetic modifier I-BET151 in a murine model and was shown to prevent insulitis in NOD mice and also led to β-cell proliferation and improved β-cell function. The drug was found to alter macrophage function most notably by affecting the expression of genes in the nuclear factor κB pathway and switching macrophages to an anti-inflammatory phenotype [79]. Further study of epigenetics could possibly help elucidate the modulatory effects that environmental factors can play in T1DM susceptibility. Epigenetic changes can also serve as early biomarkers of the disease, and epigenetic targets may be amenable to pharmacological intervention and used for T1DM prevention studies.

Preventive Interventions

Preventive strategies target individuals who are at high risk of progression to clinical disease. Adequate disease prediction is therefore critical before these strategies can be applied. Prediction of T1DM can be done by using a combination of genetic susceptibility loci and presence of autoantibodies. It is clear that there is a period of subclinical islet autoimmunity that can be detected by the presence of autoantibodies, which predates clinical disease manifestation. This phase provides a window of opportunity when prevention strategies may be tested in individuals at high risk of progressing to clinical disease. Prevention

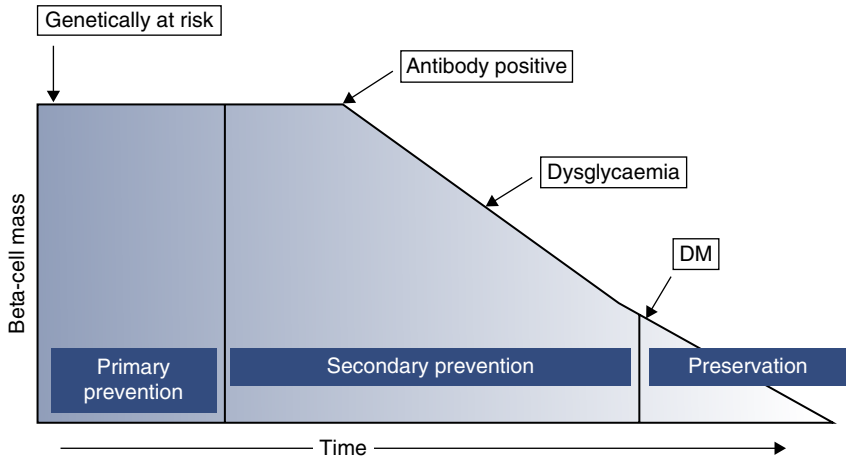

Figure 15.4 Potential time points for primary, secondary and tertiary intervention trials in T1DM [80]. *Source:* Reproduced with permission of John Wiley and Sons.

can be primary, seeking to prevent onset of autoimmunity in genetically high risk individuals; secondary, aiming to retard progression to clinical disease in those individuals with evidence of autoimmunity and preservation; or tertiary, seeking to preserve or regenerate β-cell function in patients with established T1DM (Figure 15.4).

Primary Prevention Trials

As primary prevention trials are carried out in healthy individuals with only a possible risk of development of disease, the intervention needs to be safe. Most trials have been based on dietary interventions, which seek to modify environmental risk factors that may trigger islet autoimmunity.

Dietary targets have been discussed previously (Table 15.3). Cow's milk avoidance has not been shown to be effective [61] but there is preliminary evidence from the Finnish Dietary Intervention Trial for the Prevention of T1DM (FINDIA) that removal of bovine insulin from the diet may reduce the incidence of islet autoantibodies by age 3 years in at-risk children [81]. Delayed gluten exposure does not appear to reduce the risk of T1DM autoantibodies [64]. Early data from the Nutritional Intervention to Prevent (NIP) Type 1 Diabetes study indicate that supplementation with the omega-3 fatty acid docasohexaenoic acid (DHA) produces equivocal results [82]. There are ongoing trials with vitamin D supplementation [83].

The Pre-POINT trial is investigating the effect of mucosal insulin exposure on the risk of islet autoantibody development. It aims to study the optimum dose and route on insulin administration (oral or nasal) for a further larger phase II/III primary prevention trial in at-risk children [84]. It has found a regulatory T-cell response to 67.5 mg of oral insulin.

Secondary Prevention Trials

No secondary prevention trials have shown efficacy in preventing T1DM.

Non-antigen specific: Nicotinamide, a vitamin B3 metabolite, is protective against β-cell damage in animal models of T1DM. The European Nicotinamide Diabetes Intervention Trial (ENDIT) evaluated the effects of supplemental nicotinamide in at-risk relatives of individuals with T1DM and failed to find any protective effect [85].

Antigen-specific: Insulin has been identified as the key autoantigen in T1DM and several studies have used insulin in intervention trials. It was postulated that constant exposure to the antigen might lead to a state of induced self-tolerance. The Diabetes Prevention Trial Type 1 (DPT-1) studied the effect of subcutaneous and oral administration of insulin on development of diabetes in high-risk individuals, with neither oral nor subcutaneous insulin exposure found to reduce the development of T1DM [86, 87]. A *post hoc* analysis showed that individuals with high titre of insulin autoantibodies at baseline had a delay in the onset of disease by about 5 years and that rates of T1DM onset increased when oral insulin was stopped in the intervention group [88]. This observation prompted the Type 1 Diabetes TrialNet consortium to fund another study examining the effect of oral insulin on the risk of diabetes progression in relatives of T1DM patients (NCT00419562); results are awaited. The Finnish Diabetes Prediction and Prevention Project (DIPP) failed to find an effect of nasal insulin administration on diabetes progression [89]. The INIT II study being conducted in Australia is testing the effect of intranasal insulin administration in children and young adults with risk of progression to T1DM (NCT 00336674).

Immunomodulatory agents: TrialNet is currently conducting a randomized placebo-controlled trial with teplizumab, an anti-CD3 monoclonal antibody to study its efficacy in preventing T1DM onset in autoantibody positive individuals with dysglycaemia (NCT01030861). Another study by TrialNet is looking

Table 15.3 Primary prevention trials in T1DM.

Agent/study	Intervention	Main finding	References
Cow's milk protein, TRIGR	Weaning to an extensively hydrolysed casein formula	Initial pilot study from Finnish cohort of TRIGR did show reduction in autoantibody formation but the completed study has not shown any effect on development of β-cell autoimmunity	[60, 61]
Cow's milk bovine insulin, FINDIA	Weaning infants to an insulin-free cow's milk formula	Pilot study showed reduction in autoantibody formation	[81]
Gluten, BABYDIET	Delaying onset of gluten in >12 months	No effect on islet autoimmunity	[64]
Docasohexaenoic acid (DHA), NIP	DHA supplementation in the last trimester of pregnancy or early infancy	Pilot trial showed increase in RBC DHA levels, no effect on cytokine levels	[82]
Vitamin D	Routine supplementation (400 U) versus high dose supplementation of 2000 U	Results awaited	[83] NCT00141986

at the effect of abatacept (CTLA-4 Ig) and its effect on prevention of development of T1DM in relatives at risk (NCT 01773707).

Tertiary Prevention Trials

Tertiary prevention trials are carried out in newly diagnosed individuals to try to preserve residual β-cell function. The end point of most of these studies is preservation of C-peptide concentrations following a mixed meal challenge. The autoimmune process is firmly established in individuals who develop T1DM so most of the initial agents were immunosuppressant, including prednisolone, cyclosporin and azathioprine. These agents did induce a remission phase but it was short-lived and the effect was lost when the medication was stopped. Significant side effects have limited the use of immunosuppressant agents [90].

Antigen-specific therapy: GAD is an important autoantigen. It was postulated that administration of GAD alum might modify the immune response in diabetes. While an initial phase II study on 70 children with T1DM showed a reduction in the rate of fall of C-peptide [91], two subsequent larger studies failed to show benefits [92, 93].

A randomized control trial which examined the effect of the altered peptide ligand of insulin, NBI-6024, which is designed to inhibit autoreactive T cells, did not show preservation of β-cell function [94]. Another proinsulin vaccine is also being trialled [95].

Immunomodulatory agents: In an attempt to down-regulate the primarily T-cell-mediated islet immune response, anti-CD3 monoclonal antibodies have been investigated. The humanized anti-CD3 antibodies, hOK-T3gl (Ala-Ala/Teplizumab) and ChAglyCD3 (TRX4/Otelixizumab), have been tested in humans. Teplizumab was initially studied in a randomized control trial in T1DM patients within 6 weeks of onset and led to significant preservation of C-peptide levels, even after 2 years of treatment with a reduction in glycosylated haemoglobin (HbA1C) and reduced insulin requirements [96]. The recently completed Protégé study failed to meet its primary outcome of HbA1C < 6.5% and insulin use of <0.5 U/kg/day but the data from the complete study have shown that those treated with the full-dose 14 day regimen had relative preservation of C-peptide levels compared to the placebo group [97, 98]. A phase II trial reported on the use of Oteliximab in 80 patients with new-onset T1DM and showed that insulin requirements were lower in the treated group, even after 48 months of a single course of therapy [99]. This was translated into a larger phase III study DEFEND-1 but the trial failed to meet its primary end point of change in C-peptide and the follow-up study, DEFEND-2, has been suspended. The significantly lower dose used in this study with a view to avoiding side effects may be one reason for the poor effect [100].

Rituximab (antiCD20) is a potent B-cell-depleting antibody and has been tested in a phase II trial of newly diagnosed T1DM subjects. While the 1 year data were encouraging and showed reduced rate of fall of C-peptide, better HbA1C and reduced insulin requirement in the treatment group, the 2 year follow-up showed that the groups had merged and there was no difference in the area under the curve of C-peptide [101, 102].

Abatacept (cytotoxic T-lymphocyte antigen-4 [CTLA-4]) did demonstrate an initial reduction in β-cell decline but the rate of decline of C-peptide was parallel in the abatacept and the control group after 6 months. Long-term parenteral administration of the trial drug limits its widespread applicability [103, 104].

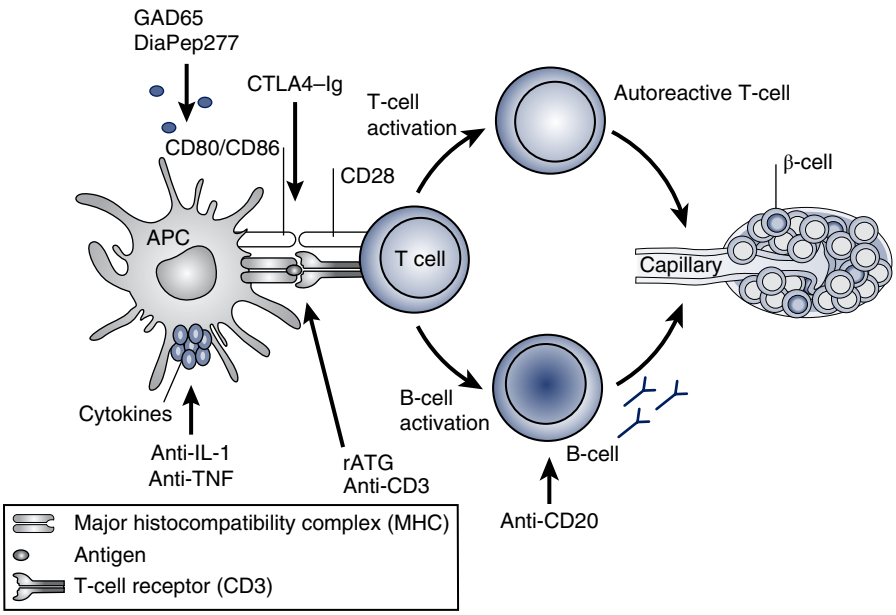

Figure 15.5 Postulated mechanisms of immunomodulatory therapies [108]. *Source:* Reproduced with permission of Springer.

Antithymocyte globulin (ATG, a polyclonal anti-human T-cell antibody), Anakinra (anti-IL-1Ra) and Canakinumab (anti-IL-1b antibody) have not been shown to be effective at preserving β-cell function [105, 106]. DiaPep277, a synthetic peptide related to heat shock protein 60 with immunomodulatory characteristics, has been shown to reduce the decline of stimulated C-peptide in adults with new-onset T1DM [107]. Imatinib mesylate, a tyrosine kinase inhibitor, is currently the subject of a trial in new-onset T1DM (NCT01781975). The various mechanisms of postulated immunomodulatory agents are shown in Figure 15.5.

In short, no immunosuppressive agent used in intervention trials has preserved β-cell function. Although studies have demonstrated short-term reductions in C-peptide decline, the results have been transient. Safety issues remain paramount with the use of immunomodulatory agents and the safety profile of these drugs must be established before they can be considered for widespread use.

Diagnosis

Diagnostic Criteria

Definitions for the diagnosis of diabetes have been developed by the American Diabetes Association and the World Health Organization. Diabetes mellitus is diagnosed by:

1) Classic symptoms of hyperglycaemia or hyperglycaemic crisis plus random plasma glucose concentration ≥11.1 mmol/L (200 mg/dL).
2) Fasting (for at least 8 hours) plasma glucose ≥7.0 mmol/L (126 mg/dL).
3) 2 hour plasma glucose ≥11.1 mmol/L (200 mg/dL) during an oral glucose tolerance test (OGTT) using 1.75 g/kg of anhydrous glucose dissolved in water to a maximum of 75 g [109].

Haemoglobin HbA1C ≥ 6.5% (48 mmol/mol) is another diagnostic criterion for diabetes in adults. The studies that support this criterion were conducted in adults, so it is not recommended for use in children [109]. Haemoglobin HbA1C ≥ 6.5% has been shown to have poor sensitivity but high specificity in the diagnosis of early T1DM in youth [110]. In the absence of clinical symptoms such as diabetic ketoacidosis or classic symptoms of hyperglycaemia, a second test is needed to confirm the diagnosis. Blood glucose (BG) meters and urine test strips for glycosuria and ketonuria can be useful screening tools in an office or clinic but the diagnosis must be confirmed by laboratory measurement of plasma glucose concentration.

Presentation

Symptoms include polyuria, polydipsia, nocturia, secondary enuresis, weight loss or lack of weight gain in keeping with poor gain in height, polyphagia, fatigue and blurred vision. Candidal nappy rash is common in infants and toddlers, while school-age and teenage girls often have vulvar/vaginal candidal infections. Symptoms are typically present for a few days to weeks but can be present for a few months. Diabetic ketoacidosis (DKA) at onset is reported in 13–80% of children with rates varying widely

around the world. This variation is associated with the background rates of T1DM, distance from the equator and inversely with gross domestic product [111]. Younger age and lower socioeconomic status are associated with higher rates of DKA at diagnosis [112].

Management and Therapies

Overview of Management

Newly diagnosed T1DM requires urgent management. Presentation may range from life-threatening diabetic ketoacidosis to milder presentations with lesser degrees of hyperglycaemia, ketosis and dehydration. Even in milder presentations, arrangements for specialist management including insulin replacement should occur within hours because of the risk of rapid decompensation, especially in younger patients.

Initial priorities are:

1) Correction of acute metabolic derangements, including diabetic ketoacidosis (see below).
2) Management as an inpatient or day-care patient according to individual needs and local practices.
3) Establishing insulin replacement therapy, which is always required in T1DM.
4) Establishing an initial nutritional plan.
5) Teaching the young person and their family/carers initial survival skills, including insulin injections, self-blood glucose monitoring (SBGM), ketone testing and hypoglycaemia management.
6) Assessing psychosocial functioning and assisting with acute psychological reactions and coping.
7) Establishing a relationship with the multidisciplinary team for ongoing care.
8) Confirming the classification of diabetes by laboratory testing (type 1 diabetes-related antibodies and C-peptide) and diagnosing and treating co-morbidities such as coeliac disease or autoimmune hypothyroidism. Where the classification of diabetes is uncertain, additional testing may be required, e.g. genetic testing for monogenic diabetes.

Longer-term goals and priorities include:

1) Optimizing insulin replacement with the aim of maximizing normoglycaemia and minimizing episodes of hypoglycaemia and hyperglycaemia.
2) Avoiding or minimizing severe hypoglycaemia (with seizure or coma) and diabetic ketoacidosis.
3) Continuing education tailored to individual family needs to optimize self-management skills, including blood glucose monitoring, insulin adjustment, nutritional plans and management of exercise, sick days, hypoglycaemia and travel.
4) Regular follow-up with a multidisciplinary team with training and expertise in the management of T1DM, including the medical specialist (pediatric endocrinologist or pediatrician), dietitian, diabetes nurse educator and mental health professional (social worker or psychologist). The primary care physician (general practitioner) is included as an active member of the team.
5) Appropriate access to the diabetes team for additional urgent or *ad hoc* advice by telephone or electronic communication.
6) Avoiding or minimizing risk of long-term microvascular and macrovascular complications, including following a recommended schedule for screening for diabetes complications.
7) Achieving normal growth and puberty.
8) Optimizing mental health and psychosocial functioning for the individual and family, with preventative and interventional strategies as needed.
9) Achieving a coordinated and effective transition to young adult services according to local practices, usually between the ages of 16 and 19 years.

Consensus and Evidence-Based Guidelines

The management of T1DM in children and adolescents has been reviewed in national and international consensus- and evidence-based guidelines. The most important are the ISPAD Clinical Practice Consensus Guidelines 2018 [113]; The American Diabetes Association 'Standards of Medical Care in Diabetes 2015' [114]; the UK NICE Guideline 'Diabetes (type 1 and type 2) in children and young people: diagnosis and management' [115]; Australian National Evidence-Based Clinical Care Guidelines for Type 1 Diabetes in Children, Adolescents and Adults 2011 [116], Diabetes Canada Clinical Practice Guidelines 2018 (https://guidelines.diabetes.ca/cpg); and Care of Children and Adolescents with Type 1 diabetes, a statement of the American Diabetes Association [117]. These are excellent reference sources and information in this chapter is consistent with these guidelines, unless otherwise stated. A variety of resources are also available for patient education [118–122].

Insulin Therapy

Insulin is essential, life-saving therapy for T1DM and is delivered subcutaneously. Ideal replacement would simulate the physiological secretion of insulin with variable basal insulin action and rapidly responsive postprandial insulin action (onset and offset) regulated accurately in response to changes in BG levels [123]. Modern insulin regimens attempt to approximate this by the use of multiple daily injections (MDI) or insulin pump therapy (continuous subcutaneous insulin therapy, CSII), both delivering insulin subcutaneously. With current technology this simulation of normal physiology is imperfect but can provide satisfactory metabolic control if well applied. It is hoped that improved insulin pump technology with

continuous glucose monitoring (CGM) and feedback automation will improve glucose control.

There are three main categories of insulin regimen for children and adolescents with T1DM [115]:

1) Multiple daily injection therapy (MDI).
2) Insulin pump therapy (also called continuous subcutaneous insulin infusion, CSII).
3) Simplified injection therapy.

The choice should be individualized for each patient and family taking into account age, family and child preference, lifestyle, activities and psychosocial factors [115, 116].

Insulin Requirements

Insulin doses vary considerably over time and between individuals, depending on the duration of diabetes, activity, diet, growth and pubertal status and insulin sensitivity. The appropriate total daily dose of insulin is that which optimizes glucose control without excessive hypoglycaemia. Typically at diagnosis, children and young people are started on total daily insulin doses of 0.5–1 units/kg/day distributed in an injection regimen as described below. Younger children tend to require lower doses while pubertal patients, those recovering from ketoacidosis or those on steroids require higher doses [117].

In the first 1–3 weeks, there is often significant insulin resistance and doses up to 2 units/kg/day or more may be required for short periods until a partial remission phase is achieved. During the remission phase, with exogenous insulin support, there can be some recovery of pancreatic insulin secretion and total daily insulin requirements may be <0.5 units/kg/day for some months. Inevitably, however, residual insulin secretion fails and long-term requirements are usually in the range of 0.7–1 units/kg/day. Puberty is a time of physiological insulin resistance and insulin requirements up to 1.5 units/kg/day or higher may be needed until the majority of growth and puberty have been completed. Insulin requirements are generally lower (up to 15% less) for the same or better metabolic control when insulin is delivered by CSII than by intermittent injections [124].

Available Insulins

A range of insulin types with different absorption profiles are available [125]. Recombinant human and analogue insulins have largely replaced animal-derived insulins. The standard concentration available is U100 (i.e. 100 units/mL). Other concentrations of insulin such as U40 or U500 are available in some places but should be considered only in special circumstances [126]. Similarly (and where available), animal-derived insulins have a very limited role.

Table 15.4 shows the range of commonly available insulins used in clinical practice in children and adolescents, with basic characteristics as per manufacturers' data [118, 123]. These characteristics are a general guide and responses will vary in individuals.

The different action profiles of insulin are generally related to the variation in the rate of dissociation of insulin from subcutaneous conjugates and the native hexameric structure to the active monomer. Manufacturers have manipulated this by alterations in amino acid structure, pH and binding additives such as zinc and protamine, which alter the rate of absorption from the subcutaneous site into the circulation. The exception to this is the long-acting insulin analogue insulin detemir [126], which is absorbed quickly from the subcutaneous site but forms a complex with albumin, which slows the release of insulin monomers into the circulation. Once in the circulation as free insulin, all insulin is identical.

New developments have aimed to improve insulin absorption/action characteristics and also to reduce variable absorption and dose-dependent kinetics (meaning that a larger dose of insulin has a longer duration of action as well as an increased magnitude of effect). Modern analogues are improved in these aspects but day-to-day absorption of insulin can still be influenced by injection technique, injection site, local injection site changes (lipohypertrophy or lipoatrophy), exercise and temperature. All insulins display dose-dependent kinetics to some extent but available data suggest that this tendency is less in insulin analogues [123].

Rapid-Acting Analogues (For Example, Insulin Lispro, Aspart and Glulisine) These are analogues of insulin produced by minor amino acid substitutions that cause the hexameric bonds to be weaker and dissociate to monomers more quickly. This reduces the absorption time and hence reduces time to onset of action and duration of effect compared to regular human insulin. They are the most suitable insulin to use as a pre-prandial insulin in multiple daily injection regimens and are widely used. They are also suitable as additional doses to correct hyperglycaemia and are used in insulin pumps. Data are inconsistent whether rapid-acting analogues show clinical advantages over regular insulin; high quality studies are lacking [116]. Even more rapid insulins are under development and testing (e.g. fast-acting insulin aspart {Fiasp, Novo Nordisk}).

Short-Acting/Regular Recombinant Human Insulin (For Example, Actrapid and Humulin R) These insulins are also suitable for use as pre-prandial and correction dose insulin but have a slower onset of action and longer duration than rapid-acting analogues. They have a significant role in insulin regimens in children, especially where the prandial insulin dose needs to cover a main meal and the next between-meal snack. Short-acting insulin has

Table 15.4 Recombinant and analogue human insulin preparations widely used in children with T1DM and their approximate action profiles [123].

Insulin type	Onset of action (h)	Peak of action (h)	Duration of action (h)
Rapid-acting insulin analogues: Insulin aspart (NovoRapid, Novo Nordisk) Insulin lispro (Humalog, Eli Lilly) Insulin glulisine (Apidra, Sanofi)	0.25–0.5	1–3	3–5
Short-acting insulin (regular or soluble): Insulin neutral (Actrapid, Novo Nordisk) Insulin neutral (Humulin R, Eli Lilly)	0.5–1	2–4	5–8
Isophane insulin: Insulin isophane (Protaphane, Novo Nordisk) Insulin isophane (Humulin NPH, Eli Lilly)	2–3	4–12	8–24
Long-acting insulin analogues: Insulin detemir (Levemir, Novo Nordisk) Insulin glargine (Lantus, Sanofi)	 1–2 2–4	 6–12 Relatively peakless	 20–24 20–24
Mixed insulins (less commonly used)			
Rapid/long-acting mix: Insulin aspart 30% soluble, 70% protamine crystallized (NovoMix 30, Novo Nordisk) Insulin lispro 25% plus insulin lispro protamine suspension 75% (Humalog Mix25, Eli Lilly) Insulin lispro 50% plus insulin lispro protamine suspension 50% (Humalog Mix50, Eli Lilly)	0.5	4–12	8–24
Short/Long-acting mix: Insulin neutral 30%, isophane insulin 70% (Mixtard 30/70, Novo Nordisk) Insulin neutral 50%, isophane insulin 50% (Mixtard 50/50, Novo Nordisk) Insulin neutral 30%, isophane insulin 70% (Humulin 30/70, Eli Lilly)	0.5	4–12	8–24

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been used in insulin pumps but rapid-acting analogues have superior characteristics [127]. Regular (soluble) human insulin is the preferred insulin for use in intravenous insulin infusions (see below).

Long-Acting Insulin Analogues (For Example, Insulin Glargine, Insulin Detemir and Insulin Degludec) These analogues have been developed in an attempt to improve basal insulin characteristics [126]. In T1DM injection plans, they are used in combination with rapid-acting or short-acting prandial insulins. In insulin glargine, minor modification of amino acid structure shifts the isoelectric point, making the complex less soluble and more slowly absorbed at physiological pH. Duration of action is up to 24 hours with no pronounced peak, although effects vary in individuals. A waning effect has been reported after 20 hours [128] which is less pronounced when U300 glargine is used. Glargine is frequently used as a once-daily basal insulin but may be better divided into two doses if large doses are required. A U300 version of glargine is reported to lead to a more constant profile with a prolonged duration of action compared to U100 glargine but data in children and young people are lacking [126].

Insulin detemir has a single amino acid alteration in the α chain and the addition of a myristic acid side chain to the N-terminal of the beta chain. It is absorbed rapidly from the circulation but forms equilibrium with albumin in the circulation, which slows delivery of free monomeric insulin and makes it more predictable. Detemir is most commonly used twice daily as basal insulin [123] and has been reported to have a more reproducible profile of action, less hypoglycaemia and a weight-sparing effect compared with insulin glargine [126]. Detemir and glargine are not unit equivalent, with data indicating a 27–38% higher dose of detemir required to achieve comparable glucose control [129, 130].

Both insulin glargine and detemir have been shown to be superior to NPH insulin for reducing risk of hypoglycaemia, although available (limited) studies have not shown consistent advantages in metabolic control. Practising clinicians generally favour basal analogues over NPH insulin where they are available.

Insulin degludec is another basal insulin available in some countries with a prolonged duration of action that is suggested for once daily basal administration [126]. Data in young people are currently lacking.

Intermediate-Acting Isophane (NPH) Insulin Isophane insulin has been very widely used and is still used in many countries, either as a basal insulin once daily in an MDI regimen or twice daily in MDI or simplified injection regimens. A major disadvantage is that the formulation is a suspension and thorough re-suspension is needed before each administration, which is often done incorrectly. Data show that isophane insulin is associated with greater variability in insulin action and increased risk of hypoglycaemia [131]. Unless alternatives are lacking, it is not recommended for insulin regimens in T1DM.

Premixed Insulin Combinations A variety of premixed insulin combinations containing basal and prandial insulins are available as shown in Table 15.4. These can be used in simplified twice daily insulin regimens but the flexibility to alter doses of the two components independently is lost. They may be useful where the simplest possible insulin plan is needed to assist adherence to therapy but do not generally meet the aims of near-physiological insulin replacement.

Subcutaneous Insulin Injections Regimens in use

The aim of any insulin management plan is to provide adequate basal insulin levels both day and night with the addition of appropriate doses to cover food intakes (main meals and snacks). The choice of insulin regimen and delivery system needs to be individualized and a small number of common regimens have emerged.

Multiple Daily Injections (MDI) or Basal-Bolus Insulin Therapy Modern guidelines suggest the use of a multiple daily injection plan with a combination of pre-prandial rapid- or short-acting insulin and basal insulin when deemed clinically appropriate [115, 132]. Many pediatric centres use MDI regimens as their default treatment of choice.

In a typical MDI regimen, rapid-acting or short-acting insulin is given before each main meal (usually breakfast, lunch and dinner) and basal insulin cover is provided by one or two injections of a long-acting analogue. Although there is individual variation, basal insulin typically accounts for ~30–50% of the total daily dose with the remaining amount made up by the pre-prandial doses. For basal insulin, a once daily glargine (U100) injection is typically used, given in the evening for adolescents and in the morning for pre-adolescents. This means that for adolescents there is good basal insulin action in the pre-dawn period and the waning tail of glargine is in the early evening when it can be compensated with pre-dinner insulin. In pre-adolescents, the waning tail of glargine is in the pre-dawn hours when basal insulin requirements are lowest. Data suggest that insulin glargine may be given once daily at any suitable time [128, 133]. Alternatively, basal insulin cover can be provided with insulin detemir, typically given in twice daily in children and adolescents [123]. Data suggest that higher unit doses of detemir (~27% higher) are required to achieve similar glycaemic control compared to glargine [129].

The choice of pre-prandial insulin in MDI regimens can be varied according to individual circumstances, especially food intake patterns. Rapid-acting analogues work best when most carbohydrate intake is at main meals and between-meal snacks are minimal. If young people are having substantial between-meal snacks (e.g. mid-morning or mid-afternoon), regular insulin may have a better profile of action for administration pre-breakfast and/or pre-lunch. Some adolescents choose to have more than three pre-prandial insulin doses per day to cover additional times of significant carbohydrate intake but adherence to such a routine is more difficult.

Simplified Injection Regimens These are regimens in which insulin is divided into two or three injections per day:

1) Twice daily regimen: Rapid- or short-acting insulin with intermediate or long-acting insulin (NPH or detemir or glargine) is given before breakfast and before the main evening meal. Typically ~2/3 of the total insulin is given pre-breakfast and 2/3 of each dose is intermediate or long-acting insulin. Such treatment is typically limited by a tendency to hyperglycaemia in the middle of the day and afternoon.

   Such a regimen can be custom mixed or doses delivered with separate pens or premixed insulin preparations can be used if indicated. It should be noted that manufacturers do not recommend the mixing of long-acting analogues with short- or rapid-acting insulin because the latter may be partly converted to basal insulin.
2) Three times daily regimen: Rapid- or short-acting insulin with intermediate or long-acting insulin (NPH or detemir or glargine) is given before breakfast; rapid- or short-acting insulin is given alone in the mid-afternoon or pre-dinner and intermediate or long-acting insulin is given before bed or before the main evening meal. Distributions are similar to the twice daily regimen.
3) Other insulin regimens are possible taking into account the action profiles of available insulins and the needs of individual patients but these would be expected to be used only in unusual circumstances.

Continuous Subcutaneous Insulin Infusion (CSII or Insulin Pump Therapy) Insulin delivery by CSII (insulin pump) is now a routine therapy for TIDM in children and adolescents and used by the majority of patients in many clinics. CSII is suitable for any patient provided that families and individuals can be adequately educated and adhere to recommended routines. Consensus guidelines recommend that insulin pumps be especially considered for patients with recurrent, severe hypoglycaemia, wide fluctuations in BG concentrations, suboptimal diabetes control, microvascular and/or risk factors for macrovascular complications or an injection regimen that compromises lifestyle. CSII has also been suggested as being particularly helpful to young children and infants, high level athletes, pregnancy and eating disorders [134].

Insulin pumps aim to simulate more closely the physiological secretion of insulin by providing a continuous basal infusion, which can be fine-tuned to meet physiological needs with the addition of user-activated boluses of insulin to cover carbohydrate intake and correct glucose concentrations to target levels. Additional features such as temporary basal settings and different patterns of meal dosing gives the user flexibility to adjust insulin needs. Rapid-acting insulin is most suitable for pumps and is delivered from the pump in most cases to the subcutaneous site through tubing and a synthetic or stainless steel cannula inserted under the skin and changed every few days. In some countries, pod or patch pumps are available in which the insulin reservoir and cannula are in one unit to which the controller communicates wirelessly [135]. Data from most pumps can be uploaded to clinical software to assist the user and healthcare team.

- Efficacy of pumps
  Data from individual studies suggest modest improvements in HbA1c in the range of 0.2–0.6% compared to injection therapy in children and adolescents [136, 137] but most recent meta-analytical data show little improvement in HbA1C, rates of severe hypoglycaemia or quality of life [138, 139]. These meta-analyses and subsequent reviews have highlighted the suboptimal quality of the available published outcome data [140, 141].
- Adverse events
  Some studies suggested that CSII users may have an increased incidence of diabetic ketoacidosis because of the risks of interrupting insulin delivery and no subcutaneous basal insulin bolus [142, 143] but other studies have indicated that rates of DKA may be similar or lower in patients/families who are well educated in CSII use [136, 137].

  Other potential adverse effects are problems at the set insertion sites (irritation, infection, lipohypertrophy), inadvertent set disconnection, occlusion or mechanical pump failure. Education on all of these issues is essential.
- Insulin pump settings
  Insulin delivery is more efficient with CSII compared to injections and a number of studies have reported lower insulin requirements in the order of 5–20%. When switching from MDI to CSII, it is common practice to reduce the total daily dose of insulin by 10–20% for programming the initial pump settings (or more if insulin detemir has been used as the basal insulin) [144] (Table 15.5).

Details of insulin pump dose settings and requirements have been reported [122, 145–147] and a number of general principles established. The total daily insulin dose (TDD) requirement in setting up an insulin pump is estimated using factors such as age, weight, pubertal status, nutritional intake, exercise patterns and previous doses of insulin.

Basal insulin is usually started around 40% of the TDD (range 35–50%) and can be divided equally over the 24 hour period or, more commonly, set up in the biphasic pattern to which most evolve [145, 148, 149]. This varies slightly according to age and pubertal status: infants and young children tend to have lower basal needs overnight, increasing by 10–20% from 6 to 10 am, lowering across the middle of the day and increasing by 10–20% in the early to mid-evening. Similar patterns have been observed in adolescents, except that they frequently have a stronger dawn phenomenon in which there are increased insulin requirements from pre-dawn (3–4 am), presumably because of hormonal cycles.

Bolus insulin settings can be calculated from the estimated TDD by using 'rules of thumb' [122]. The '500 rule' is used to calculate an initial insulin-to-carbohydrate ratio as follows: 1 unit of insulin will cover 500/TDD grams of carbohydrate. The '100 rule' is used to calculate an initial insulin sensitivity factor or correction factor as follows: 1 unit of insulin will lower the BG by 100/TDD mmol/L. Others have suggested that for children and adolescents the rules should use a number <500 in the calculation for ICR (e.g. 450) and >100 in the calculation for ISF (e.g. 120), at least in some age groups [145, 147, 150]. It should be stressed that these rules are designed to provide a starting point from which fine-tuning is performed based on data from BG monitoring and clinical progress. Patients will not necessarily align with theoretical factors. It is common for many young people to require a stronger carbohydrate setting to cover breakfast because of lower insulin sensitivity at this time and for younger children to require weaker correction factors overnight.

Table 15.5 Guide to initial insulin pump rate settings in children and adolescents.

Parameter	Considerations and calculations
Total daily dose (TDD) The starting point for all calculations	Estimate the required total daily dose based on current circumstances. Usually reduce current total daily insulin dose on injections by ~20% if well controlled. If current insulin dose is >1 unit/kg/day, consider reducing total daily dose for pump start by 30% or to 1 unit/kg/day (whichever gives the lowest)
Basal rates Daily basal requirement ~40% of TDD. The basal rate should change 2–3 hours before the maximum effect is required	Options: 1) Evenly divide basal requirement across the 24 hours (flat basal rate) 2) Biphasic basal pattern according to age/pubertal status (preferred): a) Infants and young children have lower basal needs overnight, increasing by 10–20% from 6 to 10 am, lower across the middle of the day and increasing by 10–20% in the early to mid-evening b) Adolescents, similar biphasic set-up except have a stronger dawn phenomenon and increased insulin requirements from pre-dawn (3–4 am) presumably because of hormonal cycles
Carbohydrate bolus: insulin to carbohydrate ratio (ICR) = number of grams of carbohydrate covered by 1 unit of insulin Use '450 rule'	1 unit of insulin will cover 450*/TDD grams of carbohydrate With subsequent adjustment it is common for a stronger ICR to be needed for breakfast
Insulin sensitivity factor (ISF), or correction factor = number of mmol/L that 1 unit of insulin will lower the BGL Use '120 rule'	1 unit of insulin will lower BG by 120*/TDD mmol/L Younger children may require (or parents may prefer) weaker ISF overnight than during the daytime
Target glucose levels	Set-up varies according to pump model Suggested target is 5–6 mmol/L
Duration of action of insulin Used in insulin on board (active insulin) calculations	Commonly set 3 hours in infants and young children and 4 hours in adolescents
Safety limits and alarms	Set-up varies according to age, insulin requirements, pump model and individual preference

This is a general guide only and individual clinical judgement must be applied. Individual adjustments are made from initial starting rates by close follow-up with the diabetes team.
Others have suggested the use of 500 in the rule for ICR and 100 in the rule for ISF from adult data [122, 123, 145].

Other key parameters to be set in the insulin pump are the duration of action (usually set at 3–5 hours; young people often 3 hours), target glucose (usually set at 5–6 mmol/L) and a variety of alarms and delivery limits. Table 15.5 shows typical initial set-up for pump parameters.

Insulin Delivery Devices and Storage

Insulin can be injected subcutaneously using insulin pens or syringes. Pens are preferred for ease of use, flexibility and accuracy when available. For certain insulins, pens are available that allow half-unit increments, which are useful when the administered dose is in the range of 0–5 units. Evidence from a recent meta-analysis suggests clinical benefit and patient preference for pens over vials and syringes [151].

Jet injectors, which force insulin through the skin using a high pressure air jet, are available for subcutaneous insulin administration. They may be useful for severe needle phobia [123] but are not generally recommended because data suggest increased variability of absorption and tissue bruising [152]. Subcutaneous indwelling catheters for intermittent insulin administration are sometimes used to aid initial adaptation in difficult cases [153] but basal analogues cannot be co-administered with rapid or short-acting insulins. The catheters should be replaced every 2–4 days to avoid local site and absorption problems. Most do not persist with these for practical reasons.

Insulin should be stored according to manufacturers' recommendations at 2–8°C for long-term storage [123]. In moderate climates, the vial or pen in use can generally be kept at room temperature without significant loss of potency for up to 4 weeks. Insulin should never be exposed to direct sunlight, left in a closed vehicle or frozen. Families should receive education on these practical issues as part of their diabetes education program.

Insulin Injection Sites and Absorption

The generally recommended sites for subcutaneous insulin injections are the abdomen, anterior and lateral aspect of the thighs, upper lateral quadrant of the buttock and lateral aspect of the arms. The abdomen is the

preferred site for pre-prandial insulin as absorption is most rapid. Absorption tends to be slower from the thighs or buttocks making them preferred for basal insulin (reviewed in [123]). Young or lean children often have minimal subcutaneous tissue in the upper arms, which increases the risk of painful or intramuscular injection so this site is often avoided. Skin cleaning or disinfection is not generally required prior to insulin injections unless there is obvious contamination.

The injection technique and needle length selected aim to deliver the insulin subcutaneously, avoiding injections that are too shallow (intradermal) or too deep (intramuscular), which will adversely affect absorption. Needle lengths from 4 to 12.7 mm are available. Young people should generally use a 4, 5 or 6 mm needle, selected according to the needs of the individual and the preferred injection technique. Slim individuals and those injecting into a limb may need to lift a skin fold, especially when using a 5 or 6 mm needle [154]. Education needs to include detailed training about injection techniques and injection sites.

Insulin Adjustment

Adjusting insulin requires understanding insulin action profiles and adequate BG profiling and needs to be done in the context of the individual's glycaemic targets, growth, puberty, lifestyle and nutritional plan. Teaching insulin adjustment is a key component of diabetes education.

In an MDI pattern, overnight and morning fasting BGLs tend to be most informative for basal insulin adjustment and 2 hour post-prandial BGLs for meal dose adjustment. Pre-prandial doses should be adjusted based on overall patterns as well as pro-actively for the circumstances of the day [132] such as food intake, exercise, current BG levels, etc. Pattern-based and pro-active adjustment should be emphasized and retrospective adjustment methods such as sliding scales have no place. Iterative adjustments are made usually in the order of 10–20% of the current dose, with adequate time to allow the efficacy of changes to be assessed.

Some individuals adjust meal insulin doses by a 'fuzzy logic' method whereby they make adjustments from their 'usual' pre-meal dose based on ambient circumstances. Alternatively, an insulin to carbohydrate ratio (ICR) and insulin sensitivity (correction) factor (ISF) can be applied to pre-prandial dose adjustments as used in insulin pumps; this functionality is supported by some BG-measuring devices. Insulin also needs to be adjusted for circumstances such as sick days, exercise and travel. Patients and families frequently find insulin adjustment challenging and this relates to the large amounts of random and unexplained variation in BGLs that occur with current management modalities.

Insulin pump settings are adjusted based on clinical review and BG patterns, often assisted by data from pump data downloads and analysis software [155]. Overall doses need to increase with age, growth and puberty and it is important that basal to bolus ratios do not become unphysiological over time [122]. Families and individuals require extensive education about pump adjustment and some never achieve a good level of comfort with adjustments, often relying on regular adjustment by health professionals. In making adjustments, iterative changes of ~10–20% are usually made in the required parameters until the desired target range glucose outcomes are achieved.

Management of Sick Days

T1DM requires special care and attention during intercurrent illness because of risks of dehydration, hyperglycaemia, ketoacidosis or hypoglycaemia. Education on sick day management should be given to include clear management plans and details on how to seek emergency assistance at any time. It has been proposed that people with diabetes may have altered immune function, increased susceptibility to infection and delayed recovery but evidence for this is lacking [156].

Intercurrent illness may be associated with hyperglycaemia or hypoglycaemia depending on the type of illness. Illnesses associated with fever and non-gastrointestinal infection are usually associated with elevated BGLs because of the release of counter-regulatory hormones and resultant insulin resistance. There is relative insulin deficiency and ketosis develops with risk of DKA if not managed appropriately. On the other hand, gastrointestinal illnesses primarily presenting with vomiting and diarrhoea (most commonly viral gastroenteritis) are associated with increased risk of hypoglycaemia related to decreased carbohydrate absorption.

The following management principles apply for sick day management in T1DM [118, 120, 156]:

1) General care: Take care of the underlying illness; seek medical advice for diagnosis and treatment of any significant illness. Simple analgesics suitable for children, such as paracetamol or ibuprofen, can be used for relief of symptoms if indicated. Strenuous exercise should be avoided if unwell. When teenagers and young adults who may predominantly look after their own diabetes are unwell, parents or carers should resume more care and supervision because the teenager may be too unwell to make appropriate decisions.
2) Measure the BG more frequently, every 2 hours initially but more frequently if the BGL is trending low.
3) Check for ketones during any illness, especially if the BGL is above 15 mmol/L. Blood ketone testing is

preferred if available but urine ketone testing is an alternative.

4) Maintain hydration. Fluid losses will be increased in situations of hyperglycaemia, glycosuria, ketonuria and/or fever. If the BGL is ≤10 mmol/L, fluids containing glucose or other carbohydrate should be given [156]. Common options are pediatric oral rehydration solutions, sports drinks or diluted fruit juice or soft drink. If the BGL is >10 mmol/L, carbohydrate free liquids are preferred in order to avoid further hyperglycaemia, for example, diet soft drinks and broth.
5) Do not stop insulin or omit regular doses; extra insulin will be needed if there is hyperglycaemia and ketosis or reduced insulin doses if BGLs are running low. See Table 15.6.

Table 15.6 shows appropriate responses for adjustment of insulin and intake of carbohydrate fluids for the range of BG and ketone levels [156]. General principles are that if there is hyperglycaemia with negative or small amounts of ketones in blood or urine, an extra 5–10% of the total daily dose of insulin should be given as rapid- or short-acting insulin. This should be repeated every 2–4 hours as needed. For hyperglycaemia with moderate to high levels of ketones, usual recommendations are to give an additional 10–20% of TDD as rapid- or short-acting insulin repeated every 2–4 hours as needed and guided by frequent BG monitoring.

For glucose concentrations that are trending low, consideration may need to be given to reducing regular doses of insulin by 25–50% as guided by frequent BG monitoring. However, enough insulin and carbohydrate intake need to be given to avoid insulin deficiency, which will lead to ketosis and risk of DKA. Severe hypoglycaemia during intercurrent illness requires the administration of the full dose of glucagon recommended for age. For milder persistent or recurrent hypoglycaemia, a mini-dose glucagon protocol has been shown to have efficacy in maintaining BGLs and reducing the risk for hospitalization. See Table 15.7 [157, 158].

Families should have clear advice not to persist with home management and to seek urgent medical advice from the specialist team if the child is very young (under 5 years), the underlying condition is unclear, families are unable or unwilling to continue home care (e.g. insufficient supplies, exhausted, don't know what to do), vomiting is recurrent or persistent (more than 2 hours, especially in young children), fluid intake cannot be maintained, BGL cannot be maintained above 3.5 mmol/L, ketones are persistent or rising and cannot be cleared, the child's general condition is deteriorating in any way or there are other complicating medical diagnoses. Given clear and appropriate education and guidelines, many milder illnesses can be successfully managed at home and this can contribute to positive feelings of autonomy for families.

Special Considerations for Sick Days and Insulin Pumps

The general principles outlined above also apply to insulin pump users. The commonest reason for hyperglycaemia/ketosis in pump users is interruption of insulin delivery due to pump cannula blockage or mechanical malfunction. Education needs to include clear guidelines for how pump users respond to hyperglycaemia. Pump users should have access to blood ketone measurement in preference to urine testing. In general, in the case of hyperglycaemia, pump users should proceed as follows:

- Check the infusion site, cannula, tubing, insulin reservoir and pump for errors; if in doubt the pump set should be changed.
- If the blood ketones are negative or small (<0.6 mmol/L) and the pump and delivery set are functioning, a correction dose can be given with the pump.
- If blood ketones are moderate or high, the pump should not be relied upon. Additional insulin should be given by pen injection, either with a correction dose as would have been calculated by the pump or by the amounts outlined in Table 15.7. Once the situation is coming under control, reliance on the pump can be resumed, including additional correction boluses as needed. Often larger correction doses (e.g. 20–50% greater) than usual are needed during intercurrent illness due to insulin resistance. All pump users need to carry a rapid-acting insulin pen with them at all times.

Most insulin pumps have the facility to engage a temporary basal rate by which the rate can be reduced or increased by a specified percentage for a specified period to assist with sick day management. For example, during a viral gastrointestinal illness, the basal rate might be run at 70% of the usual amount for 6 hours until the condition improves, with constant re-evaluation. In a febrile illness with hyperglycaemia, a basal rate of 150% could be appropriate for some hours to help deal with the insulin insensitivity; again, this process needs to be guided by constant re-evaluation, including frequent BGL and ketone monitoring.

Exercise Management

Participation in sports and exercise is important for all children, including those with diabetes. It is important for physical fitness, cardiovascular health, weight control, social integration and quality of life. Young people with diabetes should be encouraged to participate in sports and educated about how to adjust diabetes management for various sports and activities. Regular exercise increases insulin sensitivity, cardiovascular fitness and lean body mass; improves blood lipid profiles; and lowers blood pressure [159].

Table 15.6 Appropriate responses for adjustment of insulin and intake of carbohydrate fluids for the range of blood glucose and ketone levels [156].

How to calculate the amount of extra insulin on sick days				**[E] No data are available from clinical trials**		
Ketones				**Blood glucose**		
Blood ketones mmol/L	Urine ketones	<5.5 mmol/L <100 mg/dL	5.5–10 mmol/L 100–180 mg/dL	10–14 mmol/L 180–250 mg/dL	14–22 mmol/L 250–400 mg/dL	> 22 mmol/L >400 mg/dL
<0.6	Negative or trace	Do not give extra insulin. May need to consider mini-doses of glucagon (see Table 15.2) if <4 mmol (70 mg/dL) Check BG and ketones again in 2 hours	No need to worry	Increase dose of insulin for next meal if BG is still elevated	Give extra 5% of TDD or 0.05 U/kg	Give extra 10% of TDD or 0.1 U/kg. Repeat if needed
0.6–0.9	Trace or small	Starvation ketones. Extra carbohydrates and fluid are needed	Starvation ketones. Extra carbohydrates and fluid are needed	Give extra 5% of TDD or 0.05 U/kg	Give extra 5–10% of TDD or 0.05–0.1 U/kg	Give extra 10% of TDD or 0.1 U/kg. Repeat if needed
1.0–1.4	Small or moderate	Starvation ketones. Extra carbohydrates and fluid are needed	Starvation ketones. Extra carbohydrates and fluid are needed. Give ordinary bolus dose	Extra carbohydrates and fluid are needed. Give 5–10% of TDD or 0.05–0.1 U/kg	Give extra 10% of TDD or 0.1 U/kg	Give extra 10% of TDD or 0.1 U/kg. Repeat if needed
1.5–2.9	Moderate or large	High levels of starvation ketones. Check BG meter. Recheck BG and ketones. Extra carbohydrates and fluid are needed	High levels of starvation ketones. Extra carbohydrates and fluid are needed. Give 5% of TDD or 0.05 U/kg. Repeat when BG has risen	Extra carbohydrates and fluid are needed. Give 10% of TDD or 0.1 U/kg	Give extra 10–20% of TDD or 0.1 U/kg. Repeat dose after 2 hours if ketones do not decrease	Give extra 20% of TDD or 0.1–0.2 U/kg. Repeat if needed
		May need IV glucose if child cannot eat or drink. Risk of developing ketoacidosis!				
>3.0	Large	Very high levels of starvation ketones. Check BG meter. Recheck BG and ketones. Extra carbohydrates and fluid are needed	Very high levels of starvation ketones. Extra carbohydrates and fluid are needed. Give 5% of TDD or 0.05 U/kg. Repeat when BG has risen	Extra carbohydrates and fluid are needed. Give 10% of TDD or 0.1 U/kg	Give extra 10–20% of TDD or 0.1 U/kg. Repeat dose after 2 hours if ketones do not decrease	Give extra 20% of TDD or 0.1–0.2 U/kg. Repeat if needed
	There is an immediate risk of ketoacidosis if the blood ketone level is ≥3.0 mmol/L					
	Insulin treatment is needed urgently! Consider evaluation of patient at emergency department					

Source: Reproduced with permission of John Wiley and Sons.
BG, blood glucose; TDD, total daily dose.
To calculate the total daily dose (TDD), add up at the insulin given on a usual day (i.e. rapid-/short-acting + intermediate/long-acting) or sum of basal rate and boluses in a pump. Do not include additional boluses given for unexpected hyperglycaemia. High blood glucose and elevated ketones indicate a lack of insulin. 'Starvation blood ketones' are usually below 3.0 mmol/L. When the child is feeling sick or vomits, and the BG is below 10–14 mmol/L (180–250 mg/dL; see table), he/she must try to drink sugar-containing fluids in small portions to keep the BG up. When ketone levels are raised, priority is to give extra insulin, and this will be difficult if BG is low. Extra insulin may be given as rapid-acting insulin analogues or short-acting regular insulin, but rapid-acting if available is preferred. Short-acting insulin can be given intramuscularly to speed up absorption. The ketone level may increase slightly (10–20%) within the first hour after giving extra insulin, but after that it should decrease [E].

Table 15.7 Outline of the mini-dose glucagon protocol [116, 154, 155].

Rationale	For milder persistent or recurrent hypoglycaemia in situations of intercurrent illness, a mini-dose glucagon protocol has been shown to have efficacy in maintaining BGLs and reducing the risk for hospitalization
Suitability	Suitable: Mild to moderate hypoglycaemia, mild to moderate gastrointestinal illness, normal neurological status, able to tolerate oral fluids but refuses, parent or caregiver willing and able NOT suitable: Severe hypoglycaemia, severe gastrointestinal illness, severely impaired mental status and activity, unable to retain oral fluids, parent or caregiver uncertain or unable
Dilution and dosing	Glucagon is prepared according to the standard instructions (concentration of 1 mg/ml or 1000 μg/ml) Glucagon dose is then drawn up into an insulin syringe as follows: Child aged < 2 years: 2 'units' on the insulin syringe (equivalent to 20 μg glucagon) Child aged 2–15 years: 1 'unit' on the insulin syringe per each year of age (equivalent to dose range of 20–150 μg) Child aged more than 15 years: the maximum dose is 15 'units' on the insulin syringe (150 μg of glucagon)
Monitoring and response	Increase frequency of BG monitoring: e.g. half hourly × 2, hourly × 2, second hourly × 2 and then third hourly until stable A satisfactory response is a rise in glucose to 5.5 mmol/L or above by 30 minutes
Non-response and repeat dosing	If satisfactory response is not achieved, a second dose at double the initial dose should be given If hypoglycaemia recurs later (after an initial satisfactory response), a second dose can be given Non-response to a second/double dose requires transfer to hospital for assessment and likely IV fluids
Severe hypoglycaemia	If severe hypoglycaemia develops at any time, administer full dose of intramuscular glucagon and call for an ambulance

In non-diabetic individuals, exercise is associated with a reduction in insulin secretion and an increase in counter-regulatory hormones, resulting in an increase in hepatic glucose production that accurately matches skeletal muscle glucose uptake [160] allowing glucose level to be maintained in a narrow range. However, insulin concentrations in T1DM are not regulated by pancreatic secretion and glucose counter-regulation is significantly impaired, with a result that hypoglycaemia or hyperglycaemia very commonly occurs. Hypoglycaemia is a risk during exercise and for a number of hours after exercise, especially if it has been intense or prolonged (delayed hypoglycaemia [161]). Young people and their families need to be aware of the hyperglycaemic effect of counter-regulatory or stress hormones that may occur during or immediately after short, high intensity exercise. Decisions on additional insulin doses should be deferred until there has been some post-exercise rest, hydration and re-evaluation of BGLs, since BGL often falls spontaneously unless there is significant insulin deficiency at the time.

The effects of exercise in young people with diabetes are complicated and variable, depending on duration and intensity, the type of exercise (aerobic versus anaerobic), timing of exercise, fitness of the individual, stress levels, ambient BG, overall metabolic control, insulin regimen and food pattern [160]. Education needs to include general principles of adjustment of food and insulin for exercise, with the opportunity for individuals to work with members of the diabetes team to plan for specific activities and events, especially those competing at more elite levels. Strenuous exercise should be avoided if hyperglycaemia and ketosis are present.

The general principles of managing exercise are reducing insulin concentrations, increasing carbohydrate intake or both. Younger children often just need some additional carbohydrate (not covered by additional insulin) for periods of increased physical activity since exercise is not often strenuous or sustained. For older children and adolescents who engage in much higher level physical activity, insulin reduction is likely to be needed. Planning and adjustment requires additional BG testing during and after exercise to monitor responses and CGM has an important role, especially for those exercising at more intense levels.

As a guiding principle, for young people on injection plans, the rapid- or short-acting insulin dose that will be most active at the time of the exercise may need to be reduced, in the range of 25–75% according to individual circumstances (Table 15.8). Long-acting insulin may also need to be reduced for prolonged aerobic activity (e.g. by 30–50% for all-day hiking) or after significant exercise to avoid overnight hypoglycaemia, for example, by 10–20%. Individuals need to understand that significant reductions in long-acting insulin will have consequences for BG management for up to 24 hours, so compensation with additional rapid- or short-acting insulin may be needed the following day. There is

Table 15.8 Guidelines for T1DM management with exercise.

Considerations
• Duration and intensity of exercise
• Active insulin during exercise
• Blood glucose pre-exercise
• Recent carbohydrate intake
Actions
• If possible, reduce rapid acting insulin pre-exercise by 25–75%, depending on duration and intensity
• If insulin dose reduction not possible, take carbohydrate 0.5–1.5 g/kg/hour depending on duration and intensity
• For pump users, reduce basal insulin 60–90 minutes pre-exercise or remove pump
• Reduce evening insulin by 10–20% post-afternoon/evening moderate exercise
• Monitor glucose before, during and after exercise
• Avoid insulin injections in sites of exercising muscles
• Record results and modify plans

a potential risk in reducing insulin too much for exercise, since insulin-mediated glucose uptake can become impaired and hyperglycaemia, ketosis and dehydration can ensue. Children with diabetes attending camps have been reported to require overall insulin reductions in the range of 10–25%, although this varies widely according to their usual individual exercise patterns and fitness [162, 163].

Additional rapidly absorbed or slowly absorbed carbohydrate is a key component of exercise management. Guidelines for the amount of extra carbohydrate required for young people based on the type of activity are available [159]. Up to 1.5 g of carbohydrate/kg/hour may be needed for strenuous exercise. It is important also that hydration is maintained during sport.

For individuals using insulin pumps, general principles for exercise adjustment are similar. For vigorous, contact or water sports of short duration the pump can be removed for up to 2 hours before or during the exercise [160], reducing the insulin exposure only by the missed basal rate amounts. Additional carbohydrate is also a key strategy for pump users, with individual decisions about whether to not bolus or partly bolus for carbohydrate intake (unbolused carbohydrate). A very useful feature of insulin pumps for exercise is the temporary basal rate which users can reduce rates by a desired percentage for a desired duration before, during or after exercise. This is particularly useful for reducing the risk of nocturnal hypoglycaemia after prolonged or evening exercise. Pumps in combination with CGMS and predictive low glucose suspend features offer promise for assisting with exercise and post-exercise management [164].

Local Complications of Insulin Administration

A range of local complications are possible from administration of insulin via intermittent injection or continuous infusion and evidence-based recommendations are available for injections [154]. Possible complications include pain, discomfort, bleeding, bruising, infection, sensitivity reactions to insulin or delivery system components and changes to subcutaneous fat (lipohypertrophy or lipoatrophy).

For intermittent injections, cleaning or disinfection of the skin is not routinely recommended unless the site is unclean or in institutions [154]. For the insertion of insulin pump delivery sets or other indwelling cannulae, disinfection of the insertion site is required. The incidence of cannula site infection in young people, which has been reported in up to 25% of patients in a cross-sectional study [165], is not known but is a common management issue for the diabetes team. Infections are usually minor and can be treated topically but more severe infections require systemic antibiotics. Infection or inflammation can be associated with poorer glycaemic control, premature set failure and reduced patient satisfaction.

Physical and immune-mediated inflammatory changes can occur in the subcutaneous fat related to insulin administration. Lipoatrophy was formerly more common with the use of less purified and animal insulins and is now rare (<1% of T1DM subjects) with the use of human insulin and insulin analogues [166]. Lipohypertrophy is more common and has been reported in up to 48% of those with T1DM. It can manifest as a firm, rubbery lesion or can be hard and scar like. It may be easily visible or require palpation for detection [154]. It contributes to variable insulin absorption and increased BG variation and is cosmetically unsightly [167]. The main predisposing factor is repeated injection or pump set insertion in the same site with inadequate rotation; reusing needles may also be a factor. Management involves avoidance of the affected sites for 2–3 months and educating patients about widespread site rotation and avoiding re-use of needles [168].

Other Medications Used in T1DM

Insulin replacement is the treatment for T1DM in children and adolescents. There is no role for sulphonylureas or α-glucosidase inhibitors (acarbose) and data suggest these increase the risk of hypoglycaemia without assisting glucose control [115]. There is no established role for amylin analogues, GLP-1 agonists or SGLT2 inhibitors in T1DM; efficacy and safety data are lacking,

although some of these agents are the subject of ongoing research studies.

Insulin Sensitizers

Metformin is a biguanide that increases insulin sensitivity and is a first-line agent in type 2 diabetes. There has been interest in adding metformin to insulin therapy, especially in individuals with significant insulin resistance. Data in overweight/obese adolescents with T1DM adolescents suggest that the addition of metformin did not improve glycaemic control but was associated with a small reduction in insulin dose and reduced BMI, albeit with increased gastrointestinal adverse effects [169]. Routine use is not recommended but it may be considered in special circumstances. Glitazones are an alternate class of insulin sensitizer but they are neither approved nor recommended for use in children and adolescents [170].

Anti-Hypertensive Medication

Effective control of hypertension in adults with diabetes has been shown to reduce progression to renal disease and reduce vascular events, with evidence for effectiveness of angiotensin converting enzyme inhibitors (ACEI) and angiotensin receptor blockers (ARB) [171]. These agents should be considered for the treatment of hypertension in children and adolescents, with the requirement for reproductive counselling in females regarding potential teratogenic effects [172]. ACEIs should also be considered for treatment of appropriately documented persistent albuminuria in children and adolescents [172]. Studies are underway investigating their place in early use and prevention [173].

HMG Co-A Reductase Inhibitors (Statins)

Statins reduce major cardiovascular events in adults with diabetes [174]. If dyslipidaemia in children and adolescents with T1DM is not adequately responsive to improved metabolic control and lifestyle measures, statins should be considered for those over 10 years of age [132, 175]. Studies have shown equivalent short-term efficacy and safety to that seen in adults, although long-term data are lacking. Pregnancy counselling is essential for post-pubertal girls because of the teratogenic risk.

Blood Glucose Monitoring

Self-monitoring of blood glucose (SMBG) is an essential component of T1DM care. It allows:

1) Recognition of BG patterns to facilitate day to day and longer term adjustment of insulin dosing.
2) Recognition of immediate issues with BG concentrations (hypoglycaemia or hyperglycaemia) that require action.
3) Safe and effective management of diabetes during particular situations, such as illness and exercise.

BG monitoring can be achieved by intermittent fingerstick measurements and a portable BG meter or by the use of continuous glucose monitoring (CGM) systems. Those using CGM systems must also have a portable BG meter as a confirmatory tool when necessary. While the development of non-invasive (e.g. transdermal) or long-term implantable BG monitoring systems has long been a goal, these are not yet a practical reality.

Intermittent Fingerstick Monitoring

BG monitoring should be performed four to six times per day with additional tests depending on individual and daily circumstances [115, 176]. There is good evidence that the frequency of BG testing correlates with better glycaemic control and reduced risk of hypoglycaemia [177–179].

The most informative times for routine BG measurements are on waking after the overnight fast and before and 2 hours after meals [176]. It is also recommended that monitoring be done periodically between midnight and 4 am, especially after insulin adjustment or high activity days. BG monitoring is also advised before exercise, driving or operating machinery [180] and at increased frequency during intercurrent illness to guide management. Insulin pump users sometimes do more BG testing, especially those who test before all carbohydrate intake.

Most glucose meters use electrochemical methodology based on the glucose oxidase method. Many have data memories and can be downloaded with result analysis. A variety of models and brands of BG meters is available, with industry standards requiring that 95% of readings be within 15% of the reference standards [176]. The usual testing site is the finger (lateral aspect of tip), although alternate site testing (e.g. forearm) has been shown to be feasible and to give similar readings [181], except in situations where the BGL is rapidly changing. A variety of fingerstick devices are available with depth adjustable lancet. The minimum depth required to obtain an adequate drop of blood should be used to minimize tissue trauma and fingerstick sites should be rotated widely to avoid callus formation and loss of fingertip sensation. The lancet should be changed frequently (recommended each time but at least daily) to reduce risks of tissue trauma and infection [118]. Training in the use of BG meters is important: significant inaccuracies can arise from poor technique and operator errors [182].

Children and adolescents, especially when young or having adherence difficulties, should have the performance and recording of BG testing supervised as appropriate for the individual. Because of the onerous requirements

of T1DM management, there is a common tendency for some to omit, minimize or falsify BG testing or recording, with adverse consequences [183].

Continuous and Flash Glucose Monitoring

Continuous glucose monitoring systems (CGMS) have become available in which a subcutaneously placed probe makes frequent measurements of subcutaneous tissue glucose by the glucose oxidase method and converts this to a plasma glucose equivalent [138]. The probe (sensor) can remain *in situ* for up to 7 days. CGMS can be used retrospectively and blinded to analyse BG patterns or used live to inform immediate diabetes management. While technology is evolving, there are still limitations related to sensor lag, absolute point reliability, durability and patient acceptability. CGM devices are not substitutes for SMBG meters, which are still required to check calibration or corroborate critical BG measures.

CGMS show promise of improved glycaemic control and hypoglycaemia detection and management, either with injection regimens or in association with insulin pump therapy. Uptake rates are currently low in the paediatric population with identified barriers being cost, insertion pain, skin irritation and alarm fatigue [138, 143]. It is hoped that future technologies will overcome these issues.

Insulin pumps coupled with CGMS systems have become available, being the first steps towards fully automated closed-loop systems in which insulin delivery decisions are made by the system. Current models commercially available in some countries either suspend insulin for up to 2 hours when a low glucose threshold is reached and the user does not respond to alarms (low glucose suspend) or suspends insulin temporarily before hypoglycaemia occurs according to a predictive algorithm (predictive low glucose suspend). Studies have shown reduced hypoglycaemia with these systems, especially overnight [184, 185]. Clinical studies are underway on a number of systems with fully automated closed-loop insulin delivery, with promising results [186]. A major challenge is the ability of closed-loop systems to control post-prandial glucose rises related to lags in glucose sensor performance and subcutaneous insulin absorption [187]. An alternate strategy showing promise is a bi-hormonal pump (capable of delivery insulin and glucagon) coupled with a CGMS system for fully automated algorithmic glucose control [188].

Flash glucose monitoring systems rely on technology similar to CGM, the main difference being that glycaemic information is provided only on request. These systems currently do not have alarms and are not linked automatically to insulin pumps.

Ketone Measurement

Ketone measurement is an essential component of T1DM care. In normal physiology, the emergence of ketones in blood and urine occurs in response to fasting as energy metabolism switches from glycogen/glucose dependence to the alternate energy source of free fatty acids and ketone bodies. In T1DM diabetes, significant ketone concentrations indicate insulin deficiency and this is usually in the context of hyperglycaemia with impending risk of diabetic ketoacidosis if not managed appropriately.

It is recommended that urinary or blood ketones are measured [176]:

1) During uncontrolled hyperglycaemia, for example, two consecutive BG concentrations above 15 mmol/L, even if not unwell.
2) During any intercurrent illness, but especially with hyperglycaemia and symptoms suggesting impending ketoacidosis, such as abdominal pain, vomiting, dehydration, drowsiness or rapid breathing.

Blood ketone testing measures concentrations of ß-hydroxybutyrate, the major serum ketone of relevance in diabetes. Home meters are available that measure this with a separate strip to that used for glucose testing. Urine ketone testing measures acetoacetate, which is less useful but still informative if blood ketone measurements are unavailable. There is a lag in ketone changes in blood being reflected in urine and blood ketone measurements have greater efficacy [189, 190].

Families and young people with T1DM need specific education about the measurement and interpretation of ketones and how to respond with additional insulin administration and fluids (Table 15.6) [118].

Emerging and New Therapies

Biological Systems

As T1DM is characterized by loss of β-cell mass, cell-based therapies have focused on replacing β-cell shortage, preserving remaining β-cells and helping β-cell regeneration. The challenges in most of these therapies have been countering the innate autoimmunity and the risk of immunological attack against the β-cells [31].

Stem Cells Stem cells have the capacity of unlimited self-renewal with the capability to differentiate into multiple cell types. They offer the hope of regenerating new β-cells to replace the ones lost due to immunological destruction. Immunomodulatory characteristics of stem cells have also been used to counter the innate immune response and help preserve residual β-cell mass and allow β-cell regeneration [191] (Figure 15.6). There are various types of stem cells:

1) Embryonic stem cells (ESC's) are isolated from the inner cell mass (ICM) of the human blastocyst. ESCs can differentiate into any cell type. This ability to differentiate into mature cell types *in vivo* and *in*

vitro is used to try to generate functional insulin producing cells (IPCs). Most successful protocols have tried to recapitulate *in vitro* the normal process of development of pancreas as it would be in an embryo. This is achieved by the use of growth factors that guide the cells through the intermediate stages of pancreatic differentiation [192]. The major problems have been generation of mature β-cells with the capability of glucose sensing [193]. Recent studies have described development of more efficient protocols leading to formation of IPC's, which release insulin in a manner comparable to adult islets and reverse diabetes in murine models [194–196]. Major limitations to the use of ESC's include ethical issues, tumorigenic potential and vulnerability to immune attack [197]. Two of these issues may potentially be addressed by encapsulation devices (Figure 15.7), which allow for easy monitoring and retrieval of these cells in case tumour formation and also masks them from the immune system [198].

2) Induced pluripotent stem cells (iPSC's) are another type of pluripotent stem cells generated by reprogramming somatic cells, with properties similar to ESC's. iPSC's have been developed by reprograming dermal fibroblasts from patients with T1DM [199]. Insulin-producing cells developed from mice derived iPSC's have been shown to reverse diabetes in NOD mice [200]. The generation of efficient IPC's is far from achieved and these cell lines also run the risk of mutagenesis and tumour formation
3) Cord blood stem cells (CB-SC's) have immunomodulatory capabilities that induce Treg cells leading to maintenance of self-tolerance. These properties have been studied in human trials by transfusion of autologous cord blood in new-onset T1DM patients to preserve residual β-cell function. Although these trials have shown improvement in Treg levels, they have failed to demonstrate improvement in C-peptide or reduction in insulin requirement [201–203].
4) Haematopoietic stem cells (HSC's) induce immunological tolerance. In the first report of haematopoietic stem cell transplantation in T1DM, mobilized autologous HSCs with cyclophosphamide, G-CSF, high-dose cyclophosphamide and rabbit antithymocyte globulin (ATG) were used in the conditioning process [204]. The latest analysis has reported a 30 month follow-up of 23 patients of whom 12 have had continued insulin independence. Significant side effects such as nosocomial pneumonia and oligospermia have been reported, limiting its use as a safe universal therapy. A recent trial from Mexico has reported on the use of a simpler and less toxic protocol using fludarabine. Over a 34 month median follow-up insulin independence has been described in 44% of enrolled subjects with minimal associated adverse events. While still the only stem cell based therapy yet shown to be effective, the risks associated with transient myelosuppression are significant and limit the widespread applicability of HSCs [191].
5) Mesenchymal stem cells (MSCs) are multipotent cells localized in several tissues including cord blood, bone marrow and adipose tissue. They have unique abilities of immunomodulation by downregulating T, B and NK cells, enhancing immune tolerance by Treg stimulation and their ability to release anti-inflammatory cytokines [191]. MSC injection into NOD mice has been shown to reduce the degree of insulitis and prevent the development of diabetes

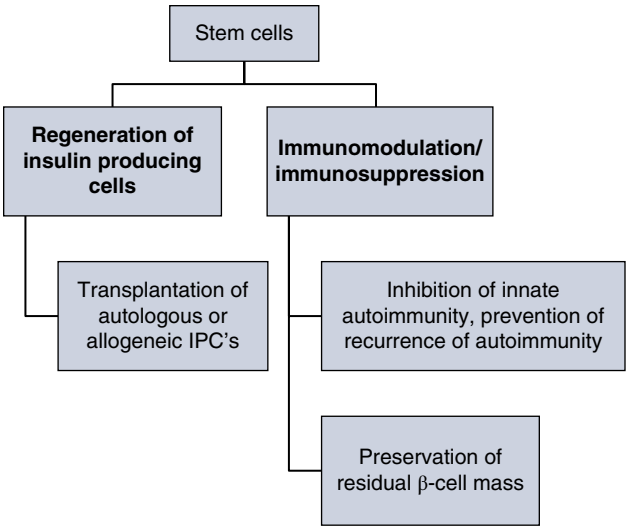
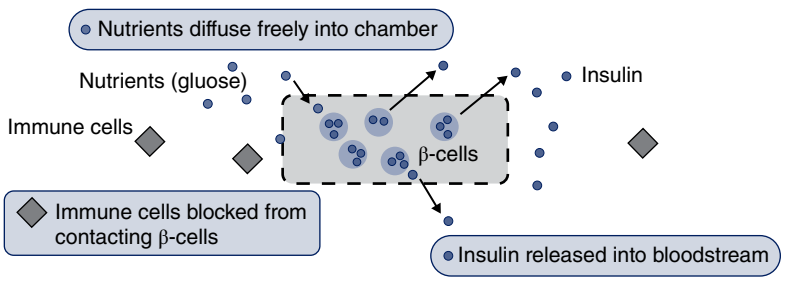


Figure 15.6 Stem cell strategies for T1DM.

Figure 15.7 Macroencapsulation device [194–197].



[205]. A randomized control trial evaluated the effect of MSC infusion on β-cell function in newly diagnosed subjects with T1DM. It showed relatively preserved C-peptide in the treatment group with decreased peak and AUC of C-peptide in the control group at 1 year of follow-up [206].

Further studies are needed to determine the safety profile of stem cell therapies and risk of oncogenicity especially associated with the use of ESC's and iPSC's. While the use of CB-SC's and HSC's appear to be safe, the immunological mechanisms of SCs need to be elucidated to understand the mechanisms behind the SC effect. Finally, the effects seen in most studies have been modest and larger and longer-term studies are needed before they can be brought to routine clinical practice [191].

Pancreatic Transplant Pancreatic transplant aims at replacing lost β-cell mass and restoring normoglycaemia. It requires lifelong immunosuppression and is therefore currently only offered under certain specific conditions to adults with T1DM. The American Diabetes Association (ADA) has specified the conditions in which pancreatic transplant may be offered [207].

Three transplant categories exist [207]:

1) Simultaneous pancreas and kidney (SPK) indicated in diabetic patients with end-stage renal disease who have had or plan to have a kidney transplant.
2) Pancreas after kidney (PAK) indicated in diabetic patients with end-stage renal disease who have had or plan to have a kidney transplant.
3) Pancreas transplant alone (PTA), indicated in
   a) Diabetic patients with frequent acute severe metabolic complications such as hypoglycaemia, ketoacidosis, labile diabetes in spite of best medical management.
   b) Patients who have incapacitating clinical and emotional problems with exogenous insulin therapy.
   c) Patients with consistent failure of insulin-based management to prevent acute complications.

In most situations SPK is offered to patients with T1DM and diabetes associated end-stage renal disease but PTA can also be offered in situations of 'brittle diabetes', severe hypoglycaemic unawareness and high risk of secondary diabetes complications [207, 208].

The latest report from the International Pancreas Transplant Registry shows that more than 26,000 pancreatic transplants have been performed worldwide between 1966 and 2011. About 80% have been SPK transplants and 8% PTA [209, 210]. SPK transplants are more common as these patients are already committed to immunosuppression following a kidney transplant and they therefore incur only an extra added surgical risk for a dual procedure. New technologies such as pumps and CGM appear to have reduced the numbers of patients experiencing severe hypoglycaemia, thus limiting the number of patients who might require PTA.

Owing to improvement in surgical techniques, better immunosuppression and graft surveillance the half-life of pancreatic transplants has increased to 7–14 years. Patient survival rates have also improved significantly to >96% at 1 year post-transplant and >80% at 5 years [211]. 5 year pancreatic graft survival rates (indicated by insulin independence) are now 71% (simultaneous pancreas and kidney transplant), 65% (pancreas after kidney transplant) and 58% (pancreas transplant alone) as reported by the IPTR [211]. More than 80% of all pancreatic recipients receive tacrolimus and mycophenolate mofetil for maintenance therapy with most protocols moving away from steroid based therapy [212].

Data suggest that there may be improvement in pre-existing diabetes-related microvascular complications in transplant recipients. Retinopathy may worsen initially with acute normalization of glycaemia but it appears to improve over the longer term. Diabetic nephropathy, neuropathy and macrovascular disease have also been shown to improve [211, 213].

Islet Cell Transplant Although outcomes of pancreatic transplant have improved, it remains a major surgical procedure with attendant risks and mortality. Islet cell transplantation is a simpler, less invasive method of β-cell mass replacement. A major advance in islet cell transplantation came with the Edmonton protocol published in 2000, which used steroid-free immunosuppression and showed results of 100% insulin independence at 1 year of follow-up [214]. The 5 year follow-up results of the same group and a larger multicentre trial using the same protocol were less encouraging with only 10% of patients maintaining insulin independence [215, 216]. However the majority of participants persisted to have some C-peptide production leading to improvement in glycaemia and reduction of severe hypoglycaemic events. Islet cell transplantation may thus be offered to a select group of T1DM patients with labile glycaemic control and high risk of metabolic complications such as hypoglycaemic unawareness.

Islets are isolated from the donor pancreas by intraductal injection of a collagenase enzyme. This is followed by density gradient islet purification, which enables procurement of a small volume, highly enriched islet extract. This islet preparation is maintained in culture for 24–72 hours before release and clinical transplantation. The islet extract is then injected by a percutaneous transhepatic route into the portal vein [217, 218].

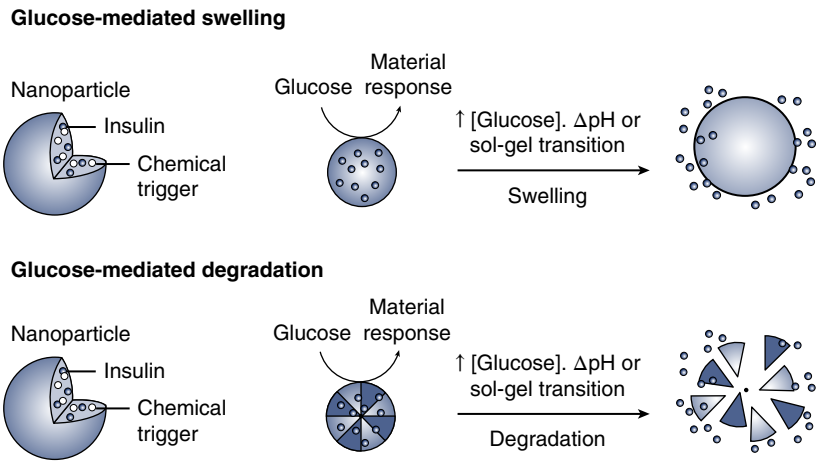


Figure 15.8 Glucose-responsive insulin delivery devices [222]. *Source:* Reproduced with permission of Springer.

The Collaborative Islet Transplant Registry (CITR) has reported on 677 transplants conducted from 1999 to 2010 of which 575 have been islet alone and 100 islet with kidney transplant. Insulin independence at 3 years after transplant improved from 27% in the early era, to 37% in the mid and to 44% in the most recent era [219]. Most major centres are now reporting 5 year insulin independence rates of 50–70% with a very low mortality rate of 1.3% [218]. These improved results have been attributable to better islet isolation and purification procedures and improved transplantation protocols. Most centres have now incorporated anti-inflammatory drugs such as etanercept and anakinra in their protocols. The use of anti-apoptotic inhibitors and GLP1 agonists including exenatide and liraglutide is also being investigated. Immunosuppression protocols have also advanced over time with use of T-cell-depleting agents such as anti-CD3 and -ATG and the addition of mycophenolate mofetil to maintenance [217]. Donor availability remains a critically limiting factor with two to four donor pancreas per patient often required. Newer encapsulation techniques have the potential to protect transplanted islet cells from the recipient's immune system and increase islet mass survival. ADA guidelines recommend islet cell transplant as a last resort for patients in whom severe hypoglycaemia persists despite optimal medical therapy [220].

Smart Insulins Smart insulin-based therapies involve the automatic release of insulin in response to increases in BG concentration. This system aims to mimic physiological insulin release and therefore must have a rapid response to glucose changes with release of biocompatible insulin [221]. This would facilitate tighter glycaemic control while minimizing the risk of hypoglycaemia.

Smart insulins consist of two components, a glucose-sensing particle and an insulin delivery device. Nanoparticle formulations using glucose-responsive molecules have been engineered that detect fluctuations in BG concentrations and respond by releasing the insulin cargo through material degradation, disassembly or swelling [222] (Figure 15.8).

The three most common glucose sensing mechanisms that have been used for such an assembly are glucose oxidase, glucose-binding proteins and glucose-binding small molecules. The glucose oxidase enzyme system has been the one most commonly used for glucose sensing and has been used by incorporation in chitosan-based microgels. It is based on the enzymatic conversion of glucose to gluconic acid in biological solutions. This process leads to a dip in the pH of the microenvironment of the complex that results in a rise in insulin solubility and consequent insulin release but the insulin release kinetics have been found to be somewhat slow and unpredictable [221, 223].

Concalvin A is the most commonly used glucose binding protein and is used as an injectable conjugate that dissociates under increased glucose concentration and leads to glucose mediated insulin release. Host immunological responses remain a concern with its use [222].

Phenyl boronic acid (PBA) is a small glucose sensing molecule that functions by changing its physical properties on glucose binding. A hyperglycaemic state would lead to swelling of the material and transition to a soluble form with subsequent insulin release. Return to normoglycaemia re-establishes the gel state of the molecule and stops insulin release. The major issue with the use of PBA is its requirement of a high pH (>8.4) to function, which is much higher than physiological pH [222]. Most research in this subject had been limited to *in vitro* experiments; however recently *in vivo* trials in animal models have been published [224].

Mechanical Systems

Closed-loop insulin delivery systems are rapidly evolving. They rely on continuous subcutaneous insulin delivery (pump) responsively linked to a method of continuous glucose sensing (CGM). The physiologic lag imposed by peripheral interstitial glucose sensing and subcutaneous insulin delivery (Figure 15.9) necessitate that the algorithms used are predictive rather than simply responsive of glucose changes. There are a variety of closed loop systems in phase III trials and close to commercial launch. Advances in this field are on several fronts: pharmacologic, ultra-fast-acting insulins; software development, better predictive algorithms that learn and adapt to individual patients; and hardware development such as glucose sensing probes co-located with insulin delivery catheters.

Dietary Considerations

Insulin is a prandial hormone. First phase insulin response under physiologic conditions is related to the effect of ingested carbohydrate and gluconeogenic substrates upon portal glucose levels. In T1DM the matching of insulin delivery to diet is the most significant contributor to good metabolic control. The complexity imposed by non-physiologic subcutaneous delivery of insulin means that even under the best possible conditions there is always a significant delay between peak insulin concentration and peak portal glucose concentration (Figure 15.9). In order to mitigate these delays, there has been an emphasis on dietary intake of complex carbohydrates with low glycaemic index that result in a slower prandial rise in portal glucose but the notion of physiologic post-prandial glucose control with the administration of subcutaneous insulin delivery remains illusory.

The key aim in managing diabetes in childhood and adolescence is to allow optimal growth and development. Thus dietary recommendations are consistent with this aim and aligned to those of otherwise healthy pediatric populations. These include that 50–55% of total energy intake should be from carbohydrate, 15–20% from protein and <35% from fat [226]. Not surprisingly in the context of diabetes much of the focus has been on carbohydrate intake.

Measuring Carbohydrate Intake

The history of diabetes care is littered with various means of assessing carbohydrate intake. These have ranged from 10 g portions, 15 g exchanges or serves, glycaemic index, glycaemic load and most recently the food insulin index [227]. All methods have worked to varying degrees over time but since used historically with varying insulin types and regimens, it is difficult to undertake direct comparisons to determine comparative efficacy. Use of glycaemic index has probably been the most robustly studied with a low GI diet being associated with a 0.55% reduction in HbA1C over 12 months in randomized controlled trial conditions [228].

Increased use of MDI and CSII regimens has resulted in an increased interest in less restrictive or 'free' diets. This necessitates a strategy of dynamic assessment of carbohydrate intake to allow insulin adjustment – carbohydrate counting associated with either CSII or flexible dose MDI. An older program of flexible short-acting insulin dosage for carbohydrate content from Germany in the 1980s [229] was re-popularized in Europe and then in the UK under the banner of DAFNE ('Dosage Adjustment For Normal Eating'). While the DAFNE program initially showed some promise in adult

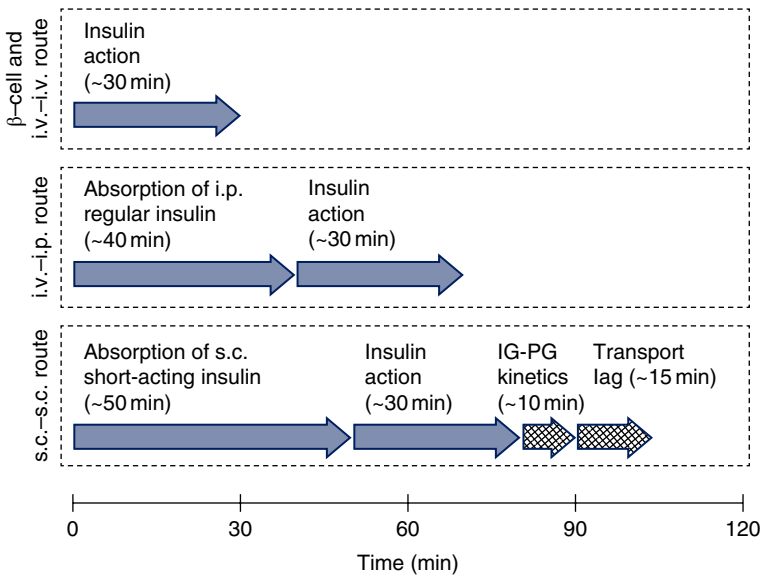


Figure 15.9 Schematic describing the physiologic delay between insulin release, binding to insulin receptor and activation of glucose transporter system of portal insulin delivery and non-physiologic delay by intraperitoneal or subcutaneous rapid-acting analogue insulin delivery. The normal physiologic delay with β-cell insulin release is 30 minutes. In systems of intraperitoneal insulin release combined with intravenous glucose sensing the delay is 70 minutes. Under the most common circumstance of subcutaneous insulin delivery, the delay is 90–100 minutes [225]. *Source:* Reproduced with permission of John Wiley and Sons.

patients (with a mean reduction in HbA1C of 0.5% over 12 months from a baseline of 9.4% [230]), these results have not been replicated in children. The large multicentre pediatric KICk-OFF Study in particular showed no impact of a DAFNE intervention upon HbA1C in a pediatric cohort [231, 232]. A recent meta-analysis of carbohydrate counting in T1DM in both adults and children concluded that carbohydrate counting did not significantly improve HbA1C concentrations [233].

Automation does not appear to be particularly helpful in this regard either. There are very few studies of 'advanced' BG meters that allow for carbohydrate counting in MDI regimens. A recent systematic review showed variable outcomes with only four studies looking at automated bolus calculators with HbA1C as an outcome in patients on MDI regimens. Although some showed improvement in HbA1C, none included children [234]. Two more recent studies of both children and adults showed improvements in HbA1C of only 0.16–0.17% [235, 236], increments that are not clinically meaningful and less than the Hawthorne effect of most insulin trials [237, 238].

Some centres have had persistently good clinical outcomes with a variety of approaches including a more restricted approach to diet [239] or alternatively a semi-restricted approach with some carbohydrate counting [240]. It is apparent that for all methods, patient ability and adherence have always remained the most determinative factors [230], with non-adherence over time remaining the norm [241].

Protein and Fat Intake

Traditionally protein and fat have been considered as 'free foods' that did not require insulin coverage. This notion has been challenged by several studies showing improved post-prandial glycaemia in patients using CSII who were allowed protein and fat when injecting bolus insulin [242]. Whether these post-prandial improvements lead to improvements in overall metabolic control over time remains an open question.

An alternative approach has been to adopt a low carbohydrate, high fat/protein diet [243]. There are numerous anecdotal reports of efficacy in terms of BG control but there are no systematic studies to date and the macrovascular safety of these diets is unknown.

Special Diets

Coeliac disease occurs in 0.6–16.4% of children with diabetes and is usually asymptomatic. The only recognized therapy for coeliac disease is a gluten-free diet [226]. Gluten-free carbohydrates may have a higher glycaemia index than gluten-containing carbohydrates with a concomitantly increased challenge to control post-prandial glycaemia. There is some preliminary evidence that a gluten-free diet in the context of coeliac disease may be associated with a reduced risk of diabetes-related kidney disease [244, 245].

Exercise

Physical activity is recognized as being an important component of health in children. Guidelines for physical activity have been developed by many countries to provide direction about the amount of exercise that is needed to support optimal health with a general recommendation of at least 60 minutes of moderate to strenuous activity per day for children aged 5–18 years. These recommendations apply equally to children with diabetes who have the potential for even greater benefit of regular physical activity. A number of studies have found that many children and adolescents with T1DM are not meeting activity targets [246]. Participation in physical exercise normalizes the child's life, enhances self-esteem, improves physical fitness, helps to control weight and may improve glycaemic control. Regular exercise increases insulin sensitivity, cardiovascular fitness and lean body mass, improves blood lipid profiles and lowers blood pressure. A recent systematic review with meta-analysis of physical activity interventions in children and adolescents with T1DM showed a modest improvement in a number of factors including HbA1c, body mass index and lipid profile [247]. Unfortunately, concern about activity-induced hypoglycaemia may restrict some children's ability to participate fully in exercise.

Skeletal muscle initially uses local glucose and then muscle glycogen as energy sources during exercise. Glucose is transported into muscle cells by the glucose transporter, GLUT4. Both insulin and exercise increase GLUT4 expression and transport resulting in a reduction of BG. In those without diabetes, insulin secretion is then downregulated but in diabetics exogenous insulin cannot be downregulated so the risk of hypoglycaemia increases [248]. Glucose responses to exercise vary in relation to the intensity and duration of the activity. Mild to moderate aerobic exercise reduces BG acutely and if >30 minutes in duration, increases risk for overnight hypoglycaemia, particularly if performed in the afternoon or evening. Intermittent, intense and prolonged moderate intensity exercise can all result in increased catecholamine production, which transiently increases BG [160]. Knowledge of these responses and reflective analysis from intermittent or continuous glucose data helps to guide actions aimed at minimizing both hypoglycaemia and hyperglycaemia with exercise [160, 249, 250].

Models of Care Delivery

In the pediatric diabetes literature, there has arguably been disproportionate attention paid to individual aspects of diabetes care and less paid to overall integrated

models of care that encompass education, medical care and psychosocial support. The emphasis will shift from an early focus on education to a later focus on psychosocial support during the course of care for a pediatric patient. In a pediatric context the whole family needs to be supported, sometimes with as much support given to the primary caregiver as to the patient. Rates of diabetes distress and mental health impairment are significant both in patients and their caregivers, particularly mothers [251].

Team Structure

Most care for children and adolescents with diabetes occurs within the context of a multidisciplinary team that includes medical, nursing, dietetic, social work and psychological expertise [252]. These teams are mostly found within the context of centralized tertiary pediatric healthcare facilities. Some teams focus upon initial inpatient care while others maintain an outpatient ambulatory care model [253]. Access to inpatient care for acute medical crises such as diabetic ketoacidosis and severe hypoglycaemia is required. Established and formalized channels of communication with other medical specialities that may be involved with unrelated health issues are critical.

The degree to which various team members act autonomously as compared to a more traditional medically controlled model vary between countries and cultures. No model of multidisciplinary team care has been shown to be superior, with centrally controlled models being potentially limited by a narrowness of focus and models with greater autonomy of team members having potential issues with harmonization and consistency of messages given to patients and their families. In many teams the boundaries between team members are blurred, with team members sharing expertise in many areas ranging from insulin adjustment to psychosocial support. Such cross-competency may be beneficial in terms of depth of expertise or problematic in terms of role confusion. Regardless of type, the success of teams universally depends upon transparency of structure and clarity of purpose for individual team members [254].

Team Philosophy

The initial post-diagnostic period can be a traumatic and bewildering time for many families. There is an enormous emphasis placed by families on the initial messages that they receive, with word-perfect recall of initial consultations being cited many years later. Thus any apparent inconsistency or incongruity of educational message may be both overidentified and overemphasized, all the more so when families are receiving messages around shared content from multiple specialists each with their own background and conceptual frameworks.

While doctors, nurse specialists and dietitians may think they are providing a consistent message, families may instead focus upon small nuanced differences in how that message is delivered and explained. In order to minimize distress and confusion, it is vital then that there is agreement within teams as to their key messages and how they should be communicated. Such agreement is predicated on an understanding of varying profession-related perspectives and mutual respect. In particular clinical goals need to be agreed.

Studies from the Hvidovre Group on various international diabetes care centres have shown that the best clinical outcomes are achieved by those centres in which there is unanimity of purpose, particularly with respect to metabolic targets [254]. Such unanimity requires active team management with regular meetings, professional development and review of clinical practices. Even in this idealized environment, it needs to be recognized that not all team members are equally effective communicators and not all family members equally receptive, particularly in the case of separated parents. After a clinical encounter it is common to blame recipients when messages appear not to have been transmitted. Patients and their families may not pay attention if they are in a state of psychosocial distress or there are intercurrent mental health issues but healthcare providers are often ineffective communicators and equally culpable [255]. Limiting the number of issues discussed at any one time, consistency and repetition of message and subsequent follow-up are all useful strategies in improving communication.

Finally there is often undue emphasis placed upon 'education' *per se* ('That non-adherent adolescent just needs more education…'). There may be some instances in which more education is required but in most cases maladaptive behaviour does not result from a lack of knowledge. Rather such behaviours occur due to variations in conceptual reasoning (e.g. adolescents focussing on the immediate term and minimizing longer term risk), social chaos, poor mental health or irrational emotive health belief systems (e.g. extreme parental fear of hypoglycaemia). The additional contribution of dysglycaemia-impaired mood and cognition has recently been highlighted [256]. Clinicians and caregivers attempt to support children and adolescents to engage in an unrelenting daily repertoire of required behaviours perceived by patients to be painful, messy, restrictive and antithetical to usual age-appropriate mores. Thus it is helpful to regard ongoing management of diabetes in children and adolescents in the context of applied behavioural paediatrics rather than correcting a seeming knowledge-deficit.

Ages and Stages and Therapeutic Regimens

There are a variety of insulin types and delivery regimens in diabetes therapy. Most have evolved in the last one to two decades but the great advances in metabolic outcomes occurred earlier. Repeated observations from randomized controlled trials and large unselected patient cohorts have failed to demonstrate that any one insulin type or regimen is consistently superior to others [257]. Diabetes care centres have shown varying clinical outcomes using the same regimens [258] and similar clinical outcomes using varying regimens [259, 260]. Moreover, even within the one centre, not all patients respond and adapt equally to all therapies [261]. The skill in contemporary diabetes management is choosing the optimal insulin type and regimen for an individual patient in a particular context. Overarching issues of equity of care and access to potentially limited resources will also be factors to consider.

The greatest patient variable to consider is the developmental age and stage of the patient. The vulnerabilities and resources required to support a toddler differ hugely from those of an adolescent. While the metabolic targets are the same for all pediatric age groups, the ways of obtaining these goals vary considerably. In young patients, the emphasis of initial diabetes education will be placed upon their caregivers. Subsequent focus may be upon diet and support at school. Children will require their own education as they grow older. Older primary school-aged children may have a greater emphasis upon schools, with an emerging need for support around sports and social activities. Adolescents will usually themselves be the primary focus of initial education and may require less support in school but increased support around sport, social activities and risk-taking behaviours. Some children find that one particular therapeutic regimen suits their needs throughout while others may find that change is required as varying life influences come into play.

Transition

Transition practices from pediatric to adult care vary considerably. Some centres care for both adult and pediatric patients in one institution so no transition occurs; others may transition relatively early in mid-adolescence to a 'young adult' clinic. The commonest practice is probably the transition to coincide with completion of secondary schooling and/or attaining the age of 18 years. All models acknowledge the critical importance of maintaining an appropriate standard of care in determining medium- and long-term health outcomes. There are distressing reports of up to 25% of patients discontinuing adult care within 2 years of transition from paediatric services. Despite this, there is no universally agreed optimal model of transitional care with case management, shared care and truncated models all being reported. Internet-based support strategies have also been described [262].

Therapeutic Goals

In a landmark study by the Hvidovre group that assessed medical, psychosocial and environmental determinants of clinical outcome in a multinational context, two aspects were dominant. These were the effectiveness of communication between parents and child and agreed metabolic targets [251]. Although the Hvidovre study was the first hierarchical analysis of the importance of targets and goal setting compared to other clinical factors, the critical nature of metabolic goals *per se* had been recognized for many years [263–265]. The importance of agreement of metabolic targets occurs both at patient/family and diabetes care team levels, with those families and teams showing the greatest unanimity of purpose and goal having the best outcomes [266].

Metabolic Targets: Glycosylated Haemoglobin

When glucose reacts non-enzymatically with one or both N-terminal valines of the β-polypeptide chains of adult haemoglobin, a Schiff base is formed, which is subsequently converted to 1-deoxyfructose. The haemoglobin molecule is said to be glycosylated. One of the forms of glycosylated haemoglobin is haemoglobin A1C (HbA1C) [267]. The average lifespan of a red blood cell is 120 days; hence HbA1C is reflective of BG control over the previous 3 months with normal values ranging from 4.0 to 6.0% (20–42 mmol/mol). If red blood cell lifespan is decreased due to a haemoglobinopathy or affected by other factors such as blood loss, blood transfusions, anaemia or high red cell turnover associated with chronic liver or renal disease, there will be less time for glycosylation to occur and HbA1C levels may be artefactually lowered [267]. Terms such as 'high glycators' and 'low glycators' have been used to describe those individuals in whom genetic, environmental or constitutional factors have interfered with the usual relationship of mean serum glucose concentrations and HbA1C [268].

Various methodologies for measuring HbA1C include immunoassay, high-performance liquid chromatography, capillary electrophoreses and enzymatic methods; all can be variably affected by confounding factors. Certified laboratories are standardized against the results of the Diabetes Control and Complications Trial (DCCT). The major US, European and international diabetes bodies have decided to change reporting of HbA1C to International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) units, which are expressed in mmol/mol rather than as a percentage.

Table 15.9 Metabolic guidelines stipulated in the 2104 Guidelines of the International Society for Pediatric and Adolescent Diabetes [176].

Level of control	Ideal (non-diabetic)	Optimal	Suboptimal (action suggested)	High risk (action required)
Clinical assessment				
Raised BG	Not raised	No symptoms	Polyuria, polydipsia and enuresis	Blurred vision, poor weight gain, poor growth, delayed puberty, poor school attendance, skin or genital infections and signs of vascular complications
Low BG	Not low	No severe hypoglycaemia	Episodes of severe hypoglycaemia (unconscious and/or convulsions)	Episodes of severe hypoglycaemia (unconscious and/or convulsions)
Biochemical assessment[a]				
SMBG PG[b] mmol/L (mg/dL)				
AM fasting or prepandial	3.6–5.6 (65–100)	4–7 (70–126)	>8 (>145)	>9 (> 162)
Postprandial	4.5–7.0 (80–126)	5–10 (90–180)	10–14 (180–250)	>14 (>250)
Bedtime	4.0–5.6 (80–100)	4.4–7.8 (80–140)	<4.2 or >9 (<75 or >162)	<4.4 or >11 (<80 or >200)
Nocturnal	3.6–5.6 (65–100)	4.5–9 (80–162)	<4.2 or >9 (<75 or >162)	<4.0 or >11 (<70 or >200)
HbA1c (%) (DCCT standardized)	<6.5	<6.0[b]	7.5–9.0[b]	>9.0[c]

Source: Reproduced with permission of John Wiley and Sons.
Units are in mmol/L or (mg/dL).
BG, blood glucose; DCCT, Diabetes Control and Complications Trial; and SMBG, self-monitoring blood glucose level.
[a] These population-based target indicators must be adjusted according to individual circumstances. Different targets will be appropriate for various individuals such as those who have experienced severe hypoglycemia or those with hypoglycemic unawareness.
[b] These figures are based on clinical studies and expert opinion, but no strict evidence-based recommendations are available. PG levels are given because BG meters are internally calibrated to reflect the plasma glucose level.
[c] DCCT conventional adult cohort had a mean HbA1c value of 8.9%, and both DCCT and EDIC have shown poor outcomes with this level; therefore, it seems prudent to recommend levels below this value.

The DCCT [269] highlighted the relationship between HbA1C and risk of microvascular pathology over the medium term, with an increasingly rapid rate of progression of retinopathy at HbA1c concentrations in excess of 7.5% [269]. There is little evidence for specific age-related HbA1C targets and the current recommendation by the International Society for Pediatric and Adolescent Diabetes is for a single target of <7.5% (58 mmol/mol) (Table 15.9). The DCCT though indicated that the relationship between risk of microvascular pathology and HbA1C was continuous with no recognizable point of inflection. Thus, some of the most recent guidelines such as the National Institute for Health and Care Excellence (NICE) have opted for lower HbA1C targets of 6.5% (47 mmol/mol) [270]. While other clinical outcomes such as avoidance of acute metabolic crises such as hypoglycaemia and DKA, minimizing glycaemic variation and maximizing quality of life are important, the pre-eminence of HbA1C as an outcome remains [271].

Metabolic Targets: Self-Monitoring Blood Glucose Concentrations

The HbA1C guidelines roughly equate to maintaining prepandrial SMBG concentrations between 4 and 8 mmol/L. The limitations of these SMBG point-of-care glucose oxidase measurement techniques should be recognized: first, BG meters are not as accurate as laboratory-based methods of analysis. The current guidelines set for the accuracy of BG meters by the International Organization for Standardization (ISO: 15197:2013) ± 0.83 mmol/L of laboratory results for glucose concentrations under 4.2 mmol/L and ± 20% of laboratory results at glucose concentrations of ≥4.2 mmol/L. Second the cut-off values of '4' and '8' are arbitrary. Hypoglycaemia can be defined in a number of ways: according to presence of symptoms, according to hormonal changes or according to changes in cognition (Table 15.10). The notion of trying to maintain BG concentrations between 4 and 8 mmol/L should be seen as useful guidelines rather than immutable values.

Table 15.10 Glucose concentrations and physiologic responses [271, 272].

Glucose concentration (mmol/L)	Physiologic response
4.4–4.7	Insulin concentrations start to decrease
3.6–3.9	Start of counter-regulatory 'stress response' (glucagon, cortisol and adrenaline concentrations start to rise)
2.8–3.1	Symptoms of hypoglycaemia, hunger, impaired cognition
<2.6	Biochemical diagnostic cut-off value for a child

Acute Complications

Hypoglycaemia

Hypoglycaemia is the most common acute complication of T1DM and is recognized as a major physiological and psychological barrier to achieving improved glycaemic control [180, 273]. Consequences can range from minor inconvenience to seizures, coma or death [274]. Hypoglycaemia or fear of hypoglycaemia is a frequent cause of anxiety in individuals and their families and often changes diabetes behaviours, resulting in tolerance of hyperglycaemia and suboptimal metabolic control in preference to risk of hypoglycaemia [275].

The common definition of hypoglycaemia in young people is usually a BG concentration of ≤3.9 mmol/L [180, 273] but the threshold for hypoglycaemic symptoms varies between individuals and in the same individual over time so a practical definition needs to include BG concentrations approaching this level with symptoms of hypoglycaemia indicating the need for intervention.

As BG falls, the counter-regulatory response includes release of glucagon, catecholamines, cortisol and growth hormone. The glucagon response is frequently impaired or lost early in the course of T1DM [276]. Children and adolescents frequently show counter-regulatory responses at BG concentrations higher than adults [277]. In situations of chronic hyperglycaemia or poor control, symptoms may be experienced at glucose concentrations higher than would normally not be considered hypoglycaemic. On the other hand, in circumstances of repeated hypoglycaemia, counter-regulatory responses may not occur until lower BG concentrations because of sympathoadrenal adaptation, resulting in a situation of hypoglycaemia unawareness [278].

Symptoms of hypoglycaemia can be categorized as:

1) Autonomic or adrenergic, including shakiness, tremor, pallor, sweating, hunger, tachycardia and palpitations.
2) Neuroglycopenic, including fatigue, headache, drowsiness, poor concentration, confusion, speech difficulty, erratic behaviour, vision disturbance, seizures and coma.

Initial symptoms are usually adrenergic followed by neuroglycopenic symptoms as glucose falls further. Responses to hypoglycaemia are age-dependent in children; infants and young children often cannot self-report, making observation of signs of greater importance.

Impaired hypoglycaemia awareness relating to blunting of autonomic responses has been shown to be precipitated by antecedent single or repeated episodes of hypoglycaemia [279]. This leads to a situation in which autonomic warning symptoms are lost or impaired and the predominant symptoms and signs are neuroglycopenic. This reduces warning features and increases risk of severe hypoglycaemic events. Hypoglycaemia awareness can be restored in such individuals by strict avoidance of hypoglycaemia over several weeks [280].

Nocturnal hypoglycaemia is a particular source of anxiety for individuals and families, with recent studies reporting alarmingly high rates of nocturnal hypoglycaemia in children and adolescents up to 40% on any given night (reviewed in [273]). These data preceded the widespread use of insulin pumps and insulin analogues and more recent data suggest that these technologies have lowered rates of nocturnal hypoglycaemia [126, 138, 281]. Counter-regulatory responses are impaired during sleep and close to half of episodes have been reported to be undetected by parents or individuals [282], with the potential for severe or prolonged episodes to result in seizure, coma or death.

Possible indicators of nocturnal hypoglycaemia are a low BG concentration, impaired thought, lethargy or headaches on waking, restless sleep or nightmares [283]. Bedtime glucose concentrations ≤5.5 mmol/L or waking glucose concentrations <7 mmol/L have been shown to be predictive but individual variation is wide because of differing food intake, exercise patterns and insulin regimens. A pre-bed snack with carbohydrate and protein [284] has been suggested as reducing risk and uncooked cornstarch has also been proposed to have some benefit [285]. Preventive measures include regular monitoring of overnight BG concentrations, including CGMS with alarms and low glucose suspension capability [184, 185].

Severe hypoglycaemia in children is generally defined as a hypoglycaemia event with coma or seizure but in adults it also includes episodes requiring assistance from others. Since children require assistance for most episodes, this makes the distinction between mild, moderate and severe hypoglycaemia more difficult [273].

The incidence of severe hypoglycaemia seems to have declined in recent years, probably due to changes in insulin types, regimens and delivery. A review of data suggests that the rate of severe hypoglycaemia in young people has declined from 17 to 27 episodes per 100 patient years to ~6 episodes per 100 patient years in the last two decades [273, 281]. The incidence of mild hypoglycaemia is unknown, since such episodes occur on a frequent and almost daily basis for many people with T1DM. They are usually self-limited and easily managed.

Precipitants for hypoglycaemia include any situation in which there is excessive insulin effect for the ambient circumstances. These include exercise, inadequate carbohydrate consumption, excess insulin administration and alcohol consumption. Alcohol impairs hepatic gluconeogenesis, which is one of the mechanisms for maintaining BG levels, especially overnight. Risks of hypoglycaemia are greater for those with longer duration of diabetes, during acute illness, in younger children, if hypoglycaemia unawareness has developed and with aberrant self-care behaviours. Earlier studies suggested increased rates of hypoglycaemia in intensively treated patients with lower HbA1c concentrations but this does not appear to be the case with modern management methods [176, 286].

Treatment of Hypoglycaemia

Mild or Moderate Hypoglycaemia In mild or moderate hypoglycaemia when the child is sufficiently alert to take food or drink orally, treatment is by ingestion of glucose or carbohydrate containing foods, with pure glucose being the preferred treatment [180]. In general 10–20 g of glucose is given in the form of glucose tablets or gel, glucose drinks or soft sweets [118]. Twenty grams of glucose in adults or ~0.3 g/kg in a child has been reported to raise BG concentrations by 2.5–3.6 mmol/L [273] but this will be influenced by other variables including the precipitants for hypoglycaemia, age and size of child and peak of insulin action.

Treatment of hypoglycaemia with juice (containing fructose), sucrose or products containing fat (such as milk or chocolate) requires larger amounts of carbohydrate as absorption and conversion to glucose is slower. Oral treatment for hypoglycaemia should not be given if the child is not sufficiently alert to chew, swallow or protect their airway during ingestion. BG concentrations should ideally be checked 15–20 minutes after initial hypoglycaemia treatment and treatment repeated if the BG level is not rising satisfactorily. In general, following glucose treatment, a snack or meal containing complex carbohydrate and protein should be given to sustain the BG concentration, although the need for this depends somewhat on how much insulin is available. Since hypoglycaemia strongly stimulates appetite, overtreatment of hypoglycaemia is common, especially in teenagers and adults if the carbohydrate consumed is unregulated. Pump users (without a depot of basal subcutaneous insulin) often find that they need less treatment for hypoglycaemia [287] to avoid the risk of overtreatment and hyperglycaemia.

Severe Hypoglycaemia Severe hypoglycaemia requires emergency treatment. Outside hospital, treatment is given by an intramuscular or subcutaneous injection of glucagon. The recommended dose is 0.5 mg for children ≤20 kg (or 6 years age) or 1mg in those above this. Alternatively a dose of 10–30 μg/kg can be given to children [273]. An unconscious or fitting patient should receive standard emergency aid including being placed in the recovery position. Emergency services should be called but glucagon should still be administered without delay by a family member or other able person.

Parents require specific education and instruction on the reconstitution and administration of glucagon, since current preparations require mixing of powder and diluent. Stable liquid forms of glucagon are under development [288] but are not yet commercially available. Nausea and vomiting are common after glucagon administration and close attention to fluid intake and BG monitoring is required, with hospitalization for IV fluids sometimes needed. The precipitant for severe hypoglycaemia should always be considered and the need for adjustment of therapy considered in communication with healthcare providers.

In the hospital setting, glucagon may also be used but an alternative is intravenous glucose in a dose of 0.25 g/kg (maximum 25 g), using an IV glucose solution of 10–25% (10 g/100 mL to 25 g/100 mL) if intravenous access and trained staff are readily available [289]. Higher concentrations of glucose or rapid administration should be avoided because of osmotic changes and potential risk of cerebral oedema.

Sudden Death in Diabetes

Sudden unexpected death has been reported to be 4–10 times more common in people with diabetes under 40 years age than the general population [290, 291], with 8–14% unexplained and attributed to 'dead in bed' syndrome. Risks are greater in male Caucasians with higher HbA1c, higher daily insulin dose, low BMI, poorer control and greater hypoglycaemia history.

The 'dead in bed' syndrome is a rare but terrifying event in which people with diabetes, particularly adolescents, are found dead in bed the next morning after apparently having gone to bed well [292, 293]. Proposed aetiological factors are severe hypoglycaemia and autonomic neuropathy and their effects on cardiac rhythm.

Other recognized causes of sudden unexpected death in young people with diabetes are intoxication with drugs or alcohol, accidental death (to which hypoglycaemia may have contributed) and diabetic ketoacidosis and its complications [290].

Diabetic Ketoacidosis (DKA)

DKA is a serious and life-threatening disorder. Its pathophysiology and management has been covered in excellent recent reviews and guidelines [115, 294–296].

Pathophysiology

DKA is the result of absolute or relative insulin deficiency and the secondary metabolic consequences that follow. Counter-regulatory hormones are markedly increased, including glucagon, cortisol, catecholamines and growth hormone, resulting in a catabolic state. This sets up a cycle of metabolic deterioration, which results in increasing dehydration, acidosis and death.

The key metabolic derangements are [294]:

1) Increased glucose production (gluconeogenesis and glycogenolysis) with impaired glucose utilization, resulting in worsening hyperglycaemia and hyperosmolality.
2) Increased lipolysis and ketogenesis resulting in ketonaemia and metabolic acidosis.
3) Hyperglycaemia (as the renal threshold for glucose of 10 mmol/L is exceeded) and ketonaemia causing osmotic diuresis with severe loss of water and electrolytes, both intracellular and extracellular. Dehydration may be further exacerbated by vomiting.
4) Increasing insulin insensitivity from further counter-regulatory hormone release creates a cycle that worsens hyperglycaemia and hyperketonaemia.
5) Lactic acidosis from decreased tissue perfusion and/or sepsis may exacerbate acidosis.

The magnitude of specific deficits in an individual at the time of presentation depends on the duration of illness, the extent to which the patient was able to maintain fluid intake and electrolytes, as well as the content of food and fluids consumed before presentation.

Prevalence and Risk Factors

DKA may be the initial presentation of T1DM or occur in people with known diabetes due to insulin omission or intercurrent illness. There is wide geographic variation in the incidence of DKA at onset of diabetes, ranging from 15 to 70%, with increased risk in the very young and in families with limited access to medical care [297]. Failure or delay in recognition of T1DM is also often a factor and has encouraged the development of community and health professional public health education programs in an attempt to reduce DKA at presentation [298].

In children with established T1DM, the risk of DKA has been reported to be between 1 and 10% [294] per year with increased risk factors being insulin omission, previous poor metabolic control or DKA, psychiatric and eating disorders, adverse social circumstances, limited access to medical services and failure to follow guidelines for management of intercurrent illness [297]. Some reports have suggested increased risk in insulin pump users due to use of only rapid-acting insulin but recent data [142, 143] suggest that this may not be the case with best-practice modern management and education. DKA can also occur at initial presentation of type 2 diabetes, with reported rates of 5–25% [299].

The mortality rate from DKA in children is reported to be 0.15–0.3% and may be decreasing [300], with cerebral oedema accounting for 60–90% of all DKA deaths.

Definition of DKA

The biochemical criteria for the diagnosis of DKA are [294]:

- Hyperglycaemia (BG concentration >11 mmol/L).
- Venous pH < 7.3 or bicarbonate <15 mmol/L.
- Ketonaemia and ketonuria.

Severe DKA is characterized as pH < 7.1 and biocarbonate <5 mmol/L.

Management

The goals of therapy are to correct circulating blood volume and dehydration, restore BG to normal concentrations, correct acidosis and ketosis and avoid complications of treatment. Management is summarized in the flow chart in Figure 15.10.

Initial Assessment and Investigations

- Assess airway, breathing and circulation (ABC).
- Assess level of consciousness (Glasgow coma scale).
- Assess the degree of dehydration. This may be greater than clinically apparent because water moves from the intracellular to extracellular space due to the hyperosmolar state, partially masking the severity of the dehydration. It is better to be conservative in the assessment of dehydration and to review progress frequently. Data suggest that patients in DKA have an extracellular fluid volume deficit of 5–10% and clinical assessment is difficult and subjective. It is recommended that

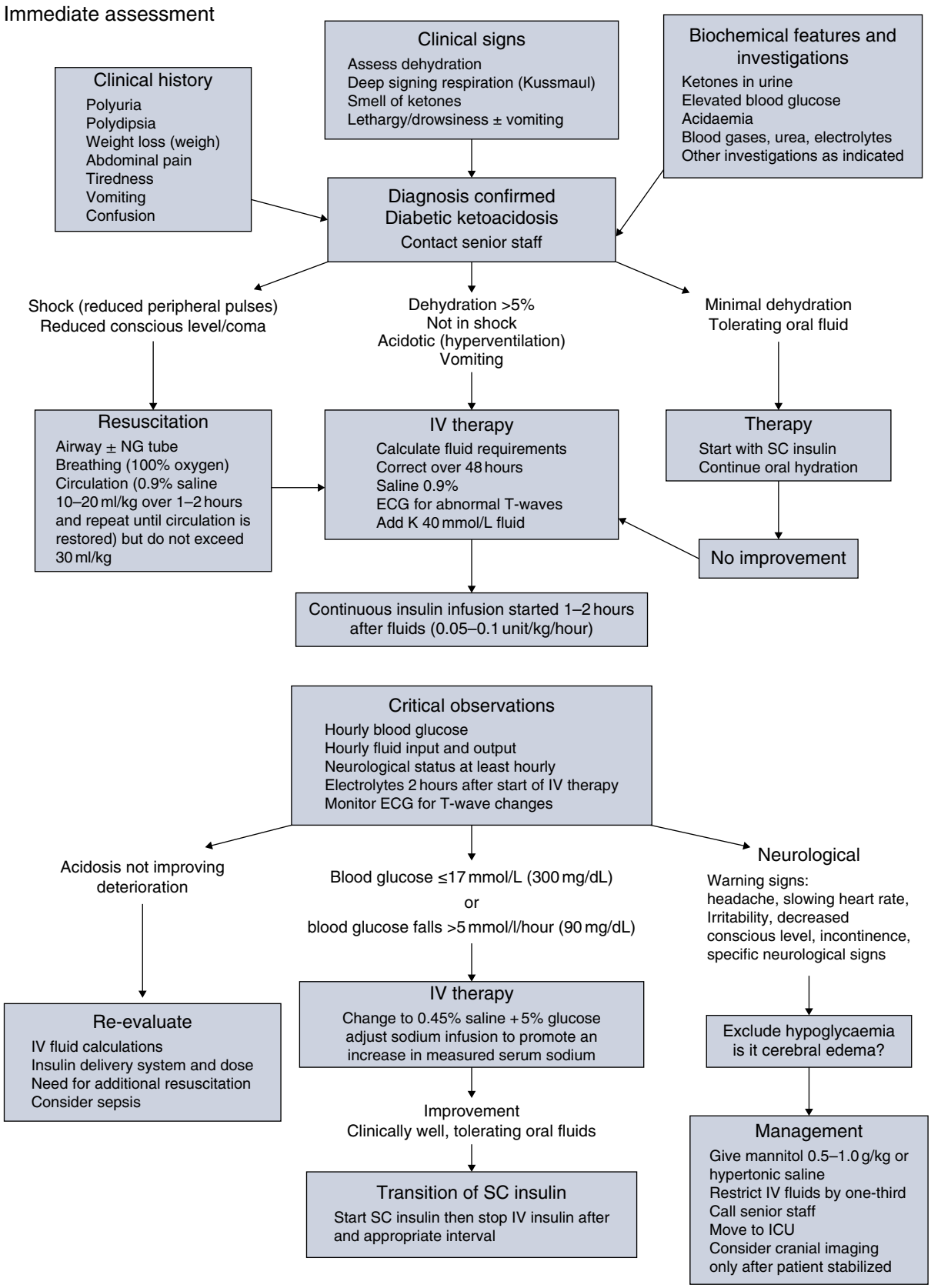


Figure 15.10 Flow chart for the management of diabetic ketoacidosis [294]. *Source:* Reproduced with permission of John Wiley and Sons.

5–7% deficit is a reasonable estimate for moderate DKA and 7–10% for severe DKA [295]. A recent study reported the median dehydration at DKA presentation to be 8.7% based on body weight before and after recovery [301].

- Measure BG and blood ketones with a bedside meter and analyse urine for ketones and glucose. Blood ketone levels are up to 0.9 mmol/L in healthy fasted individuals and are usually over 3 mmol/L in DKA [302]. Blood ketones are quantitative and measure ß-hydroxybutyrate (the major ketone in DKA) and are therefore preferred [303]. Urine testing detects acetoacetate but not beta-hydroxybutyrate and gives a delayed estimation of ketone status and may give false negative results if the strips have been exposed to air for an extended period or false positive results with certain drugs (e.g. captopril).
- Secure intravenous access, preferably with two intravenous cannulas. Arterial lines should be reserved for critically ill patients in intensive care.
- Rapid analysis of baseline blood samples for glucose, electrolytes, bicarbonate, urea, creatinine, calcium, magnesium, phosphate, osmolality, venous pH and CO_2, and full blood count. In newly diagnosed diabetes (if sufficient blood is available) add autoantibodies against insulin /GAD /IA-2/ZnT8, thyroid antibodies, TSH, and coeliac screen. Although rare, assessment for adrenal function should be performed if there is clinical suspicion of adrenal insufficiency, initially by ACTH and cortisol measurement.
- Consider a source of infection, which may have precipitated the onset of DKA. White cell count is commonly elevated due to stress and dehydration in DKA and not necessarily indicative of infection [304]. Obtain appropriate microbiological specimens if infection is suspected, for example, blood culture, urine culture and swabs.
- Obtain patient's weight.
- Institute continuous or frequent monitoring of vital signs including ECG.
- Other measures should be instituted if needed according to general management and pediatric advanced life support guidelines, such as:
  - Airway management.
  - Oxygen administration for severely unwell or shocked patients.
  - Nasogastric tube for stomach emptying/venting. Patients should generally be nil per mouth until acidosis has significantly corrected, although can have sips of water or suck ice for a dry mouth
  - Indwelling urinary catheter (consider in infants and very ill children).
  - Antibiotics for suspected sepsis, after collection of appropriate microbiological specimens.

Fluid and Electrolyte Management

- Initial fluid choice and fluid bolus

  The extracellular fluid deficit in patients with DKA is usually 5–10% with sodium deficits averaging 6 mmol/kg, potassium 5 mmol/kg, chloride 4 mmol/kg and phosphate ~1.5 mmol/kg. The goals of fluid and salt replacement therapy in DKA are to restore circulating volume, replace sodium and the ECF and ICF water to restore GFR (with enhanced clearance of glucose and ketones from the blood) and avoid cerebral oedema [294].

  If severe volume depletion or shock is present, a 10 mL/kg bolus of 0.9% sodium chloride should be given, followed by a second bolus if necessary. More than two boluses are rarely required. Avoid repeated boluses, as this may increase the risk of cerebral oedema. A contribution to decreased peripheral perfusion comes from acidosis, which will correct gradually as the acidosis is reversed. Consideration should be given to reducing rehydration rates if excessive fluid resuscitation has already been given (>20 mL/kg). Patients with mild DKA do not usually require a fluid bolus.

  Rehydration should continue with an isotonic fluid solution (0.9% sodium chloride, Ringer's lactate or PlasmaLyte) for at least the first 4–6 hours at a rate to give maintenance plus correction of estimated fluid deficit over 48 hours. Urinary losses should not be routinely added to the calculation of fluid replacement but may be necessary if urinary losses remain high.
- Potassium replacement

  At the time of presentation, serum potassium concentrations may be normal, increased or, infrequently, decreased. Since insulin promotes uptake of glucose and potassium by cells and correction of acidosis promotes the return of potassium to the intracellular compartment, serum potassium concentration may decrease sharply, once insulin commences, and early potassium replacement is necessary.

  Potassium chloride should be added to the rehydration fluid after initial boluses for volume expansion. The exception to this is if the patient is oliguric or known to have renal failure, in which case potassium therapy should be delayed until electrolyte results are known and an indwelling catheter placed. The initial KCl replacement rate is 4–5 mmol/kg/day, which usually equates to 30–40 mmol per 1000 mL of IV fluids. KCl supplementation should be withheld if the patient is hyperkalaemic until serum potassium falls to <5.0 mmol/L.

  Electrolyte results should be reassessed initially every 1–2 hours and then every 2–4 hours. Careful monitoring of the serum level and provision of adequate potassium is essential to prevent hypokalaemia and life-threatening arrhythmias.

A combination of potassium dihydrogen phosphate and potassium chloride is sometimes recommended to reduce the risk of hyperchloremic metabolic acidosis, which may sometimes delay the correction of acidosis [305]. Serum calcium needs to be monitored serially if potassium phosphate is used due to the risk of hypocalcaemia. An alternate is to use a balanced electrolyte solution such as PlasmaLyte, which has been shown to reduce the risk of hyperchloremic metabolic acidosis [306].

- Ongoing fluid management

  The aim of ongoing fluid and insulin management is to produce a fall in BG of ~2–5 mmol/L/hour [294]. The exception to this is that over the first few hours rehydration alone usually results in a larger fall, especially if a fluid bolus has been given.

  After the first 4–6 hours, fluid with a tonicity ≥0.45% sodium chloride should be used with added potassium and usually with 5% glucose. The decision to change to a hypotonic fluid will depend on state of hydration and biochemical parameters.

  Glucose should be added to IV fluids (initially 5%) when the BG falls to ~15 mmol/L (or is rapidly approaching 15 mmol/L), or if the rate of fall in BG exceeds 4 mmol/L after the first few hours. Higher concentrations of glucose in the IV fluids (e.g. 7.5 or 10%) may be required in some cases to prevent hypoglycaemia while the insulin infusion is maintained to correct acidosis. The insulin infusion rate should not be decreased below 0.05 units/kg/hour until the acidosis is corrected and ketones are cleared.

- Corrected serum sodium

  Serum sodium values require correction for the degree of elevation of BG. This is because glucose in the extracellular fluid attracts water causing dilution of extracellular solute, including sodium.

  Corrected sodium = measured sodium + 0.3 (glucose – 5.5); i.e. 0.3 mmol/L is added to the measured sodium for every 1 mmol/L of glucose above 5.5 mmol/L.

  Hyperlipidaemia, another consequence of insulin deficiency, may falsely lower the measured sodium. In this situation the laboratory will usually comment that the serum is macroscopically lipaemic.

  The measured sodium concentration should rise as that of glucose falls. Failure of the measured sodium to rise, associated with falling corrected sodium, usually indicates excess free water administration and is associated with an increased risk of cerebral oedema [307].

  If the corrected sodium falls to <140 mmol/L, 0.9% sodium chloride should replace 0.45% sodium chloride as the rehydration fluid, and the rate of fluid administration slowed by 30% if the corrected sodium continues to fall.

  If the corrected sodium is >145 mmol/L, then hypernatraemia may aggravate the hyperosmolar state produced by hyperglycaemia. If corrected sodium is >150 mmol/L, consider changing IV fluid to 0.45% sodium chloride and slowing the rate of rehydration.

  The anion gap is also useful to monitor and should improve in parallel with improvement in ketosis, acidosis and dehydration.

$$\text{Anion gap} = \text{Na} - \left(\text{Cl} + \text{HCO}_3\right)\text{mmol/L}$$
$$\text{and is normally } 10 - 14\text{mmol/L}.$$

In DKA the anion gap is typically 20–30 mmol/L, the increase being largely due to ketoacids. An anion gap >35 mmol/L, suggests significant lactic acidosis in addition [294].

Insulin Management

- The commencement of the insulin infusion should be delayed until 1–2 hours of fluid administration has been given [308] as this may help reduce the risk of cerebral oedema. The BG concentration usually starts to fall with fluids alone by increasing renal clearance.
- Insulin is commenced at 0.05–0.1 units/kg/hour by continuous infusion. One randomized trial [309] and 2 non-randomized studies [310–312] found that the lower dose of 0.05 units/kg/hour compared with 0.1 units/kg/hour resulted in similar patient outcomes with respect to BG decrease and resolution of acidosis and a more gradual reduction in effective plasma osmolality. An IV bolus of insulin at the start of therapy is unnecessary and may increase the risk of cerebral oedema and hypokalaemia [308].
- The dose of insulin should remain at 0.05–0.1 units/kg/hour until DKA is largely resolved (pH > 7.30 or bicarbonate > 15 mmol/L) or recovery of acidosis may be delayed [313]. If the rate of fall of plasma glucose is excessive after initial volume expansion (≥5 mmol/L/hour), additional glucose should be added to IV fluids.

Other Management Aspects

- Location of care

  After initial emergency management and commencement of therapy, consideration needs to be given as to where the patient is best managed. This will depend on local institutional procedures but a specialist paediatrician with training and expertise in the management of DKA and experienced pediatric nursing are required. A laboratory is required for frequent biochemical measurement.

  Generally a high dependency unit is preferred and consideration should be given for management within

a specialized pediatric intensive care unit in the following circumstances:
- Severe acidosis with initial pH < 7.1.
- Severe electrolyte disturbance (corrected sodium > 150 or < 130 mmol/L, or potassium > 5.5 or < 3 mmol/L).
- BG >50 mmol/L.
- Unexpected degree of neurologic or haemodynamic compromise or age <2 years.

For patients initially managed outside specialist paediatric facilities, close liaison with expert pediatric specialists is required and consideration should be given to retrieval to a tertiary pediatric facility if available.

- Monitoring should include:
  i) Hourly pulse, respiratory rate, BP, neurology observations and 2–4 hourly temperature. In practice, continuous bedside monitors are usually used, including ECG trace.
  ii) BG with bedside glucose meter – hourly while on IV insulin infusion.
  iii) Hourly blood ketones with bedside meter. If blood ketone strips are not available, test all urine for ketones (until negative).
  iv) Accurate fluid balance recording.
  v) 2–4 hourly (initially 2 hourly) venous pH, electrolytes (calculate the corrected sodium and anion gap), glucose and osmolality.
  vi) Reassess the state of hydration every few hours.
- If the pH is not improving over the first 3 hours of therapy,
  i) Check for problems with delivery of the insulin infusion.
  ii) Increase the insulin infusion rate if needed to 0.075 or 0.1 units/kg/hour. Some patients are quite insulin resistant due to the effects of critical illness and an extended period of hyperglycaemia and ketosis.
  iii) Consider changing the rehydration fluid from sodium chloride to PlasmaLyte, if available. PlasmaLyte has a significantly lower chloride concentration than 0.9% sodium chloride (98 versus 154 mEq/L) and higher pH (7.4 versus 5.5), preventing the tendency to hyperchloraemic acidosis with 0.9% sodium chloride. In a randomized trial PlasmaLyte versus 0.9% sodium chloride resulted in lower serum chloride and higher bicarbonate concentrations [306].

Ketamine abuse has been reported to cause severe acidosis out of proportion to the degree of ketosis [314], so consider sending a urine drug screen in teenagers with existing T1DM who present in DKA without explanation.

Recognition and Management of Any Complications

- Cerebral oedema

Cerebral oedema is the commonest cause of morbidity and mortality in DKA management. Overt cerebral oedema is reported in up to 0.9% with a mortality rate of 21–24% [295] but a greater number of children have subclinical cerebral oedema with evidence of lower GCS scores and on cerebral imaging [315].

Indicators of cerebral oedema include headache, irritability, change in neurological status including depressed consciousness, unstable body temperature, specific neurological signs, bradycardia and hypertension (late signs). Signs may be subtle and a high index of suspicion is needed. Risk factors at diagnosis or during treatment for cerebral oedema have been reported to be: greater hypocapnia or serum urea at presentation, more severe acidosis, the use of bicarbonate for correction of acidosis, a marked early decrease in serum effective osmolality, an attenuated rise in serum sodium concentration or an early fall in corrected serum sodium during treatment, greater volumes of fluid given in the first 4 hours and administration of insulin in the first hour [294, 315].

Cerebral oedema requires emergency management in intensive care. Rate of fluid administration should be reduced by one-third and a dose of mannitol (usually 0.5–1 g/kg IV over 10–15 minutes) or hypertonic saline (usually 2.5–5 mL/kg over 10–15 minutes) should be administered [294, 300] according to local protocols. Supportive measures may include intubation and ventilation and cranial imaging should be performed as soon as practical.

Bicarbonate is now very rarely used in DKA management as it is associated with paradoxical worsening of cerebral acidosis and hypokalaemia due to correcting acidosis too quickly and is not of reported benefit [316]. Bicarbonate should be considered only in cardiogenic shock due to acidosis or with symptomatic hyperkalaemia.

Transition to Subcutaneous Insulin While fluid replacement therapy is planned over 48 hours for DKA, in practice most children recover more quickly and can resume oral intake and transition to subcutaneous insulin earlier than that [317]. Oral intake can resume once there has been significant clinical improvement and dehydration and acidosis are largely resolved.

The insulin infusion can be stopped once the patient is alert and stable with BG <12 mmol/L, pH > 7.3 and bicarbonate >15 and ready to eat. Subcutaneous insulin is administered according to the regimen selected (basal and pre-prandial dose) and the insulin infusion is continued

for another 1–2 hours to prevent rebound hyperglycaemia before ceasing.

For those with new-onset diabetes, diabetes education can commence when the patient and family have sufficiently recovered to absorb information. For episodes of DKA in young people with pre-existing diabetes, precipitants for the DKA and strategies for further education, support and prevention need to be explored.

Chronic Complications

Autoimmune Complications

Coeliac Disease

Coeliac disease is more common in those with T1DM than in the general population with reported prevalence in the range of 1.6–10% [318]. Variation in rates is complicated by whether the detection of disease is through screening or by symptomatic case finding. Universal screening for coeliac disease has been recommended by a number of clinical practice guidelines and adopted by many centres around the world, although this remains controversial. Up to 85% of children who screen positive are asymptomatic, although some are found to have symptoms once screening is positive. Current screening is through IgA tissue transglutaminase antibodies (TTG).

Since IgA deficiency is more common in those with T1DM and coeliac disease, measurement of IgA to rule out IgA deficiency is needed if TTG is negative and coeliac disease is suspected [319]. In those with positive TTG, confirmation of the diagnosis with upper endoscopy with small bowel biopsy is recommended [319].

A recent systematic review of longitudinal cohort studies of screening for coeliac disease reported that in children with a median follow-up of 10 years from the time of diagnosis of diabetes, 40% were diagnosed with coeliac disease within 1 year of developing diabetes, 55% at 2 years and 79% at 5 years [318]. The effects of treatment with a gluten free diet in patients with diabetes are variable, with reports of reduction in hypoglycaemia and coeliac-related symptoms but minimal effect on metabolic control and frequent challenges with following a gluten free diet [320]. Long-term benefit in terms of reduction of risk of osteoporosis and gastrointestinal malignancy is found in symptomatic disease but less clear for asymptomatic disease.

Autoimmune Thyroid Disease

Autoimmune thyroid disease is also more common in children with T1DM than in the general population. Positive anti-thyroid antibodies (anti-thyroid peroxidase and anti-thyroglobulin) are even more prevalent, with reports of being present in 11.4% in those <12 years of age and 22.6% in those aged 12–18 years [321]. Thyroid dysfunction as defined by abnormal TSH in the setting of subclinical or overt hypothyroidism is found in 3–8% [168]. There is no strong guidance with regard to screening intervals but screening with TSH and anti-thyroid peroxidase antibodies after diagnosis and then every 2 years with more frequent intervals if symptoms of hypothyroidism are present and annual screening for those with positive thyroid antibodies is suggested [168]. The presence of antibodies is associated with a much higher risk of progression to thyroid dysfunction with a risk ratio of 25 [322]. Testing at the time of diagnosis yields a high rate of mild abnormalities in thyroid tests that normalize in the majority of patients over the next 1–2 months, particularly in those who are in DKA at the time of diagnosis [323]. Graves' disease is reported to be more common in children with diabetes but is much less common than hypothyroidism in the general population.

Addison's Disease

Up to 2% of children with T1DM are reported to have anti-adrenal antibodies with <0.5% being diagnosed with clinical adrenal insufficiency [168, 324, 325]. Those with diabetes have typical symptoms of adrenal insufficiency but in addition may present with increased frequency of hypoglycaemia and reduced insulin requirements. Given the low rates of Addison's disease, screening is not recommended but symptoms should trigger diagnostic evaluation. Testing and treatment are the same as in the general population.

Autoimmune Polyglandular Syndrome

The two major forms of autoimmune polyglandular syndrome (APS), APS-1 and APS-2, are defined by a clustering of endocrine and other autoimmune conditions. APS-1 is caused by mutations in the autoimmune regulator (AIRE) gene and is an autosomal recessive condition. Classic features are chronic mucocutaneous candidiasis, hypoparathyroidism and Addison's disease with many other less prevalent features such as autoimmune thyroid disease, chronic active hepatitis, dental enamel and nail dystrophy, ectodermal dysplasia, primary hypogonadism, T1DM and keratitis [326]. Features may develop over time so surveillance is necessary. Measurement of disease-related autoantibodies can help to identify which of the disease manifestations a patient is at high risk of developing.

APS-2 (also known as Schmidt's syndrome) is characterized by the presence of at least two of autoimmune thyroid disease, Addison's disease and T1DM. It is a multigenic disease with a significant HLA association. As in APS-1, features may develop over time [327].

Microvascular Complications

Microvascular complications of retinopathy, nephropathy and neuropathy are tightly linked in diabetes to metabolic

control. Intensive therapy with reduction in HbA1c to 7.2% was demonstrated to reduce the risk of these microvascular complications very significantly in the DCCT [269]. This study included an adolescent cohort which achieved an HbA1c of 8.1% with intensive control and experienced a similar 53–60% reduction in complications [328]. Effects of improved metabolic control are long-lasting. Long term follow-up of DCCT participants has shown that the mean 6.5 year period of improved metabolic control is associated with reduced rates of complications 18 years later, despite the loss of a difference in A1c between the two groups within 4 years of the end of the trial [329].

Retinopathy

Diabetic retinopathy is a condition of the microvasculature of the retina classified as non-proliferative (background) and proliferative. It is a leading cause of blindness and results from damage to the walls of the retinal vessels, leading to leaking and swelling and, if progressive, to neovascularization with fragile vessels prone to bleeding or retinal detachment. Retinopathy is common, with reports of 82% with mild non-proliferative retinopathy and 10.5% having proliferative or treated retinopathy after 19 years of diabetes in those who were diagnosed between 1987 and 1992. This prevalence has improved from earlier time periods (diagnosis between 1958 and 1979), when 64.3% of patients had non-proliferative and 35.7% had proliferative retinopathy [330].

Screening for retinopathy is done by seven-standard field, stereoscopic-colour fundus photography, direct ophthalmoscopy or indirect slit-lamp fundoscopy through dilated pupils or digital fundus photography. Annual screening is recommended, starting 5 years after diagnosis and at or after puberty as retinopathy is very rare before puberty. Some groups suggest initiating screening closer to the time of diagnosis and at younger ages [109, 175, 331]. Severe retinopathy can be treated with laser photocoagulation and intraocular anti-vascular endothelial growth factor therapies. Early detection of significant retinopathy allows treatment to occur before vision is threatened.

Nephropathy

Diabetic nephropathy is the most common cause of end-stage renal disease (ESRD) in Western countries and previously occurred eventually in 30–40% of persons with T1DM. Recent studies report reduction in rates of ESRD. Prevalence of ESRD after 18 years of diabetes dropped from 12% in those diagnosed in the 1960s to 4% in those diagnosed in the 1970s [332]. In Finland, a population based study in those who were diagnosed with T1DM at <30 years of age found rates of ESRD of 2.5% at 20 years of diabetes and 7.8% at 30 years [333]. Improving glycemic control and treatment of hypertension, if present, delays the onset of nephropathy and slows its progression.

Renal disease secondary to diabetes has been described initially as microalbuminuria, defined as ≥30 mg/day or ≥20 μg/min albumin in the urine. It is followed by sustained microalbuminuria progressing to overt nephropathy (macroalbuminuria) defined as ≥300 mg/24 hours or ≥200 μg/minutes albumin in the urine. Recent American guidelines have recommended using only the term 'albuminuria' reflecting that increases in albumin excretion occur on a continuum [334]. Albuminuria is defined as urinary albumin to creatinine ratio of >30 mg/g (2.5 mg/mmol).

There is increasing recognition of a significant reduction in renal function in those without albuminuria [335]. Microalbuminuria is less predictive in paediatrics, with regression in 71% and persistence in 29% of children at a 5 year follow-up [336]. Overt albuminuria is frequently accompanied by systemic hypertension and progressive impairment of glomerular filtration and typically precedes the development of ESRD by 10 years.

Progression of nephropathy can be delayed by improving glycemic control, by controlling hypertension and by treatment with an angiotensin-converting enzyme (ACEI) inhibitor or angiotensin receptor blockers (ARB). ACE treatment is recommended only for those with persistent albuminuria. Important side effects of ACEI and ARB include birth defects in developing fetuses so caution is needed when treating adolescent females. Screening for nephropathy is most easily done with a random urine sample for albumin to creatinine ratio, starting ~5 years after diagnosis [175, 337]. Albuminuria is diagnosed if two of three samples are abnormal over a 6 month period. Recognition and treatment of hypertension is important in protection from progressive renal disease.

Neuropathy

Clinically significant diabetic neuropathy is rare in children. An American population based study found that 8.2% of those with T1DM at a mean age of 15.7 years and diabetes duration of 6.2 years had signs of peripheral neuropathy after screening [338]. Early signs include loss of ankle reflexes and decreased vibration sense or touch sensation to monofilament in the great toe.

Formal cardiovascular testing detects subtle autonomic abnormalities in some adolescents with diabetes but their clinical importance is unknown [339]. Improvements in HbA1c decrease the risk of onset of neuropathy. Screening is typically recommended after puberty and 5 years of diabetes with assessment of foot pulses and reflexes and determination of proprioception, vibration and monofilament sensation along with assessment of symptoms of neuropathic pain, although the age of initiation and frequency of screening are not well defined [175, 337].

Macrovascular Complications

Cardiovascular disease is the leading cause of death in those with T1DM. The hazard ratio for cardiovascular death is 4.6 at a mean age of 36 with 20 years of diabetes duration [340]. Men and women whose diabetes commences in childhood are at high risk for macrovascular disease and women lose the protective effect of their gender. Cardiovascular events occur at a greater frequency and ~10–15 years earlier than in the general population [341]. Individuals with renal complications have an especially high risk. Other predictors of macrovascular risk and/or progression include dyslipidemia, hypertension and smoking. Strategies to reduce lifetime risk of macrovascular disease in children with diabetes include avoiding tobacco, early and vigorous treatment of hypertension and dyslipidemia and intensive glycemic control. There are no trials of statin therapy to reduce lipids and reduce cardiovascular disease in youth with T1DM. Guidelines suggest screening fasting lipids and intervening if the LDL is >2.6 mmol/L, initially with diet and then with statins if adequate improvement is not seen [175, 337].

Reproductive Complications

Adolescents with T1DM progress normally through puberty unless metabolic control is very poor. Studies have not shown hypogonadism in boys, or any significant effect on ovulation but a slight delay in menarche in girls [342–344]. As poorly controlled diabetes can significantly increase the risks of birth defects in developing fetuses, it is essential that young women with diabetes receive counselling about the importance of prenatal diabetes control and contraception [345, 346]. This counselling has been shown to be lacking in a number of studies but interventions to improve education have been successful [336].

Non-Vascular Complications of Diabetes

Cataracts

Cataracts rarely occur in children with diabetes but when present at the time of diagnosis may regress after treatment of diabetes has been initiated.

Limited Joint Mobility

Limited joint mobility (LJM), cheiroarthropathy, is caused by glycosylation of collagen in the connective tissue of skin and tendons. It manifests as inability to extend the fingers and/or wrists because of loss of skin elasticity and contraction of tendons. LJM is a sign of chronic poor glycemic control and associated with increased risk of microvascular complications.

Growth

Growth failure in children with diabetes is uncommon even with only 'average' glycaemic control but abnormality of the GH–IGF-1 axis is common. With average BG control, GH secretion is increased and serum concentrations of IGF-1 and IGFBP-3 tend to be reduced. Delayed puberty and growth failure typically occur when a child or adolescent experiences chronic, very poor glycaemic control (Mauriac syndrome). It is thought to be caused by recurrent cycles of adequate insulinaemia alternating with inadequate insulinaemia.

Skin

Necrobiosis lipoidica diabeticorum is an uncommon poorly understood complication that causes unsightly lesions that usually appear in the pretibial area. Intralesional injection of corticosteroids often results in improvement.

Brain Development

Recently researchers have highlighted the deleterious impact of T1DM upon brain development [256, 347]. These insights have occurred after years of prospective research but, in hindsight, may appear self-evident. Glucose is the primary metabolic substrate of the brain and T1DM is the illness that disrupts glycaemic stability, with glycaemic extremes being associated with changes in conscious state. Empirical evidence of the effects of T1DM upon brain development has been slow to become apparent. The central nervous system has limited accessibility for purposes of pediatric research and changes in brain morphology and biochemistry have been able to be detected only with the advent of new techniques in functional MRI and positron emission spectroscopy. The cognitive effects of T1DM are cumulative and subtle appearing mainly in tasks of executive function. These may be mitigated in part by pre-morbid cognitive ability. Changes in overall functional well-being are affected amongst other things by changes in mental health status, a frequent and compounding co-morbidity of T1DM [251]. Improved survival and increased longevity has unmasked rates of accelerated cognitive senescence [347] and this too has placed an increased emphasis upon brain ontogeny during early life years of diabetes.

MRI has shown white matter volume change in the temporal and parietal lobes with episodes of severe hypoglycaemia also affecting grey matter volume even in early childhood after relatively few years of T1DM [348, 349]. By mid-childhood (after an average diabetes duration of 7 years) grey and white matter volume changes are apparent with frontal, temporal, parietal and occipital regions of the brain affected. A history of severe hypoglycaemia or prolonged hyperglycaemia was associated with poorer outcomes [350–353]. In late adolescence (after an average diabetes duration of 12 years), white and grey matter volume changes remain with decreased biochemical markers of neuronal

density/activity and increased markers of neuronal injury [354, 355]. Acute metabolic crises such as diabetic ketoacidosis and hypoglycaemia associated with loss of consciousness are associated with acute brain volume changes and/or decreased markers of neuronal activity.

Glycaemic insults appear to be of greater consequence in the developing brain. The CNS impacts of dysglycaemia are mediated by age and duration of disease with younger age and longer duration being adverse. Thus studies of pre-school-aged children with short duration of diabetes have shown cognitive function similar to healthy controls overall but specific deficits were noted in several cognitive domains in those with a history of severe hypoglycaemia and/or high HbA1C [348, 349, 356]. By mid-childhood differences in cognitive performance between diabetic and non-diabetic cohorts become more apparent. In these older series, adverse mediators include hypoglycaemic seizures under 5 years of age and a history of DKA [357, 358].

The picture is becoming more pronounced by adolescence and early adulthood. The one longitudinal prospective case-controlled study undertaken has shown that, despite being matched for intelligence at baseline, the T1DM cohort had lost 0.3 standard deviations in full scale IQ after 12 years of disease duration. This change is mirrored by meta-analyses, which have shown decrements in full scale IQ of 0.3–0.8 SD, most specifically in areas of executive function. Population registry data from Sweden and Finland have shown suboptimal educational outcomes at both primary and secondary school levels [359–361]; greater rates of non-completion of secondary school was noted in an Australian cohort [362].

Mental Health

Adverse mental health outcomes are arguably the leading complication of T1DM in childhood and adolescence. While microvascular and autoimmune complications occur at rates of 6% or less, clinical threshold levels of affective disorders (predominantly depression, anxiety, eating disorders) occur at rates of up to 37% [251, 363, 364]. These disorders frequently persist into adulthood [365, 366]. Potential contributory factors include existential issues resulting in 'diabetes distress' and more direct neuropathological impacts of diabetic dysglycaemia [367, 368].

Prognosis

Since the DCCT, it has been recognized that chronic hyperglycaemia, as measured by HbA1C, remains the primary modifiable mediator of the long-term complications of type 1 diabetes [271]. This has resulted in continual lowering of HbA1C targets and improvements in metabolic control. Initially this came at the expense of increased rates of severe hypoglycaemia but modern diabetes treatment regimens have improved rates of hypoglycaemia [281]. While the process of improved metabolic control was accelerated by the DCCT, there had already been improvements in prognosis for patients with T1DM since the mid-1900s. This was true both for diabetes-related morbidity and life expectancy. Data from several registries show that the rates of end-stage renal failure in patients with 30 years diabetes duration fell from 31% to <1% from 1950 to 1980 [257]. Rates of diabetes related retinopathy have also fallen fivefold, even using more sensitive screening methods.

In the post-DCCT era of management, HbA1C levels have continued to fall [369, 370]. The EDIC studies have highlighted the importance of 'metabolic memory' and the legacy effects of early good metabolic control [371]. These effects include an ongoing benefit in terms of risk of microvascular pathology. It is difficult to compare risk and prognosis in contemporary youth who have had improved control from diagnosis with those diagnosed as recently as 25 years ago before to the DCCT study. It is hoped that rates of diabetes-related complications seen in current adult populations are significantly greater than those that will be seen in current pediatric patients.

Cardiovascular and cancer related deaths are the leading causes of mortality in adults with T1DM. Swedish registry data show an adjusted excess hazard ratio of mortality of 3.52 [340]. In the absence of renal disease, 7 and 20 year mortality rates respectively appear to be similar to those of a non-diabetic population [372, 373], although recent Scottish data have shown that an absence of renal disease is not a guarantee of normal life expectancy [374]. Hypertension and microalbuminuria are important modifiable predictors of diabetes-related death, myocardial infarction, significant macrovascular disease, end-stage renal disease, blindness or amputation [332]. Life expectancy has increased by ~15 years from patients diagnosed in 1965–1980 compared to those diagnosed in 1950–1964 [375]. The most recent data from the DCCT cohort indicates that there was no increase in mortality rates compared to the general population at a mean age of 55 years [376]

In pediatric and young adult cohorts DKA is the leading cause of mortality, with sudden unexpected death ('dead in bed') remaining a rare but enigmatic and concerning phenomenon [377]. Between 18 and 38 years of age, the standardized mortality ratio (SMR) has been reported as being 3.3 overall with a much greater risk for women (SMR 10.1) in Australia. The period of greatest risk was between 25 and 29 years of age [378]. These findings were similar to those seen in the UK, where the major risks factors for sudden unexpected death in early adulthood were living alone, drug abuse and mental illness [379].

Poor mental health is arguably the leading complication of T1DM during adolescence, with 10-year point and life time prevalence rates of psychiatric disorder in diabetic youth of 37–47% [251]. Poor functional outcomes at the end of adolescence have a bleak prognosis in the longer term. Prospective studies have shown that one in three adolescents with T1DM may fail to complete secondary education [362]. Lower socioeconomic status and not having a college education is in turn associated with increased risk of end-stage renal disease and macrovascular disease [380]. Depression in adolescence is likely to persist into mid-adulthood [381], is associated with metabolic syndrome [379, 382] and predicts cardiovascular disease and diabetes-related mortality [383]. Non-crisis based psychological support remains a rare event in most diabetes centres [384] and calls have been made for equal emphasis for mental health screening compared to other physical pathologies [251].

Type 2 Diabetes

Epidemiology

T1DM remains the commonest form of diabetes in children worldwide but the incidence of type 2 diabetes mellitus (T2DM) is rising in parallel with the epidemic of pediatric overweight and obesity. There are significant worldwide variations in prevalence, incidence rates and patient characteristics [170].

T2DM is most commonly seen in the second decade of life and rarely before puberty, which coincides with the peak of physiological pubertal insulin resistance. Most children have a family history of diabetes in a first- or second-degree relative [385]. Socioeconomic characteristics vary by region, with most in developed countries such as the USA belonging to lower socioeconomic and educational categories [386] and those from developing countries such as China and India belonging to the affluent class [387].

The highest prevalence is seen in African American, Native American Indian, Hispanic and Southeast Asian groups but there are wide variations depending on age, ethnicity and geography. Incidence varies from 0–330 per 100,000 person years with a prevalence of 0–5,300 per 100,000 children and adolescents. Of European countries, the Netherlands and Austria had the lowest incidence rates; the highest was in Pima Indians in the USA and in other American ethnic minorities. Prevalence of T2DM was highest in the above ethnic minority groups in the USA [388] (Figure 15.11). Data are complicated by poor reporting from developing countries and methodological variations in different studies.

Most epidemiological data about diabetes have come from the SEARCH study from the USA. The latest prevalence data from 2009 show that T2DM accounts for only about 10% of all diagnosed cases of diabetes but prevalence was higher among minority ethnic groups and the 15–19-year-old American Indian/Alaskan Native group accounted for 80% of all reported cases [389].

Long-term follow-up showed that the prevalence of T2DM has risen from 0.34 per 1000 in 2001 to 0.46 per 1000 in 2009, an increase of 30% in 8 years. Prevalence rates were higher in girls aged 15–19 years. Statistically significant increase in prevalence was found in black and Hispanic groups and in white youth. The prevalence was constant over time in Asian Pacific Islander and American Indian youth. Projections indicate that the prevalence of T2DM will increase fourfold from 2010 to 2050 [390], which has important implications on healthcare costs and long term health of the youth population.

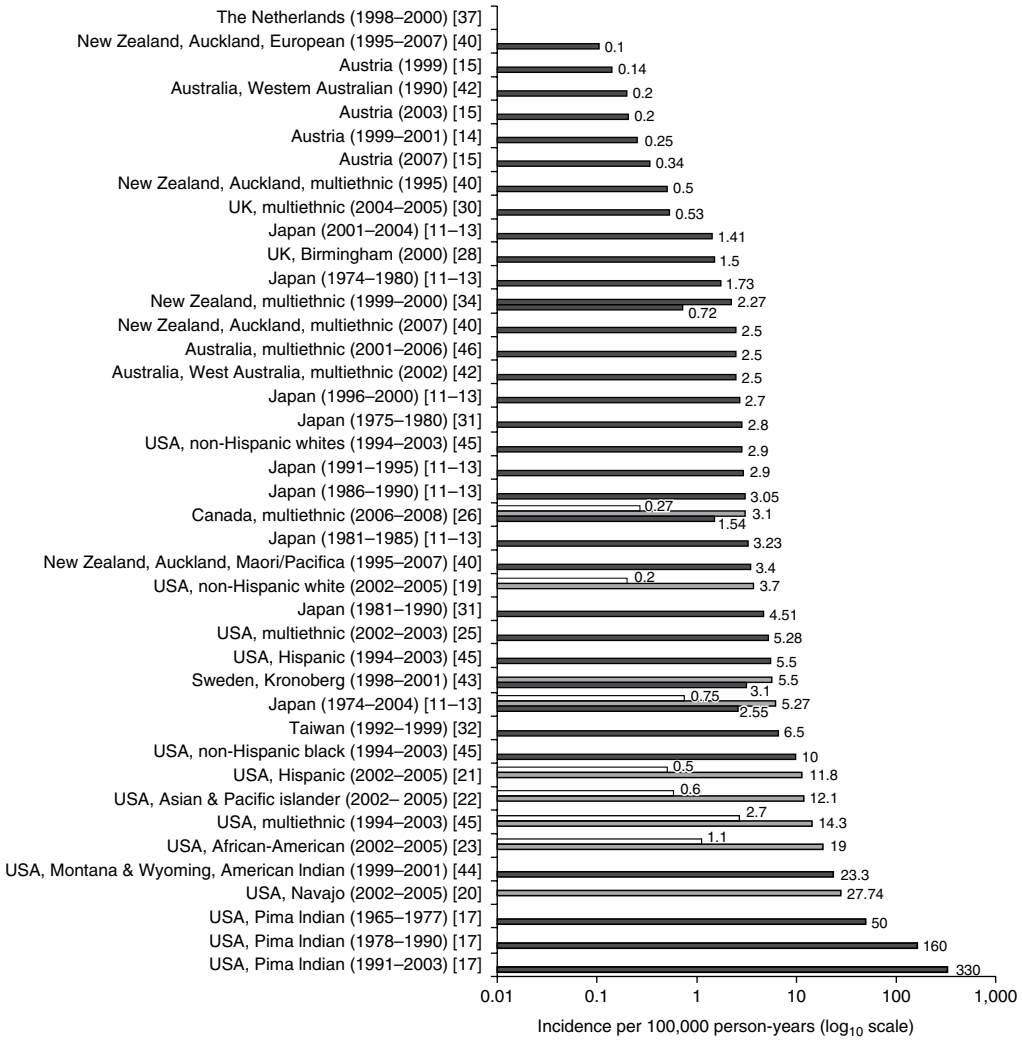
Similar high rates of T2DM have been reported from Asian countries such as Japan and Taiwan showing that more than half of all new cases of diabetes in children are diagnosed as T2DM, especially among those in the pubertal age group [170, 391], which has been attributed to a worldwide increase in obesity [392]. This was affirmed in the SEARCH study showing that 79% of all youth with T2DM were obese [393]; even though obesity rates in developed nations are plateauing [394], rates of T2DM continue to rise, which may be explained by an increase in racial minorities and the role of environmental influences.

Pathophysiology

Development of T2DM involves a progressive imbalance of glucose homeostasis. While it is understood that insulin resistance is the first feature to develop, clinical diabetes requires the second hit of β-cell insufficiency. The relationship between insulin resistance is best described by a hyperbolic function and can be quantified by the glucose disposition index, a product of insulin sensitivity and β -cell function (Figure 15.12). If there is increasing insulin resistance, there is compensatory β-cell hyperfunction to maintain normoglycaemia. When there is imbalance between these parameters and β-cell dysfunction cannot keep up with increased demands, dysglycaemia results [395].

One of the most important correlates of insulin resistance is obesity [396]. Obese children are hyperinsulinaemic and have 40% lower insulin-stimulated glucose metabolism compared with non-obese children [397]. Not all obese children develop insulin resistance. The pattern of fat distribution plays an important role and visceral adiposity plays a higher role in insulin resistance than subcutaneous fat [398].

(a)



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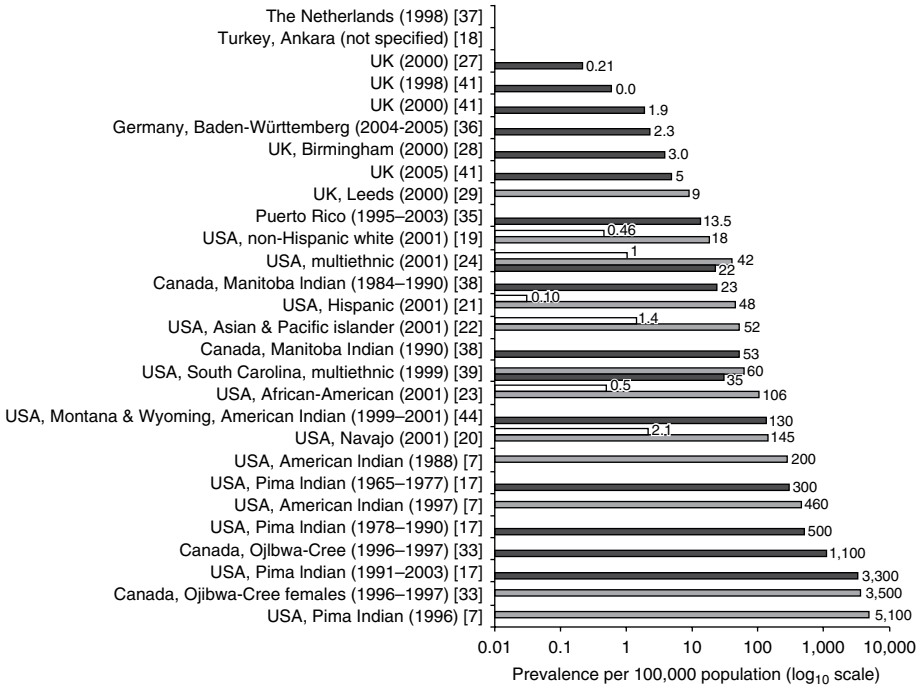

Figure 15.11 (a) Overview of the reported incidences of T2DM per 100,000 person years. (b) Overview of the reported prevalence of T2DM per 100,000 children and/or adolescents (because of considerable variations in the observed rates, incidence data are graphed on a base 10 logarithmic scale). The incidence rates were calculated for male/female populations. White bars represent children (0–9 years), grey bars represent adolescents (10–19 years) and black bars represent children and adolescents (0–19 years) [388]. *Source:* Reproduced with permission of Springer.

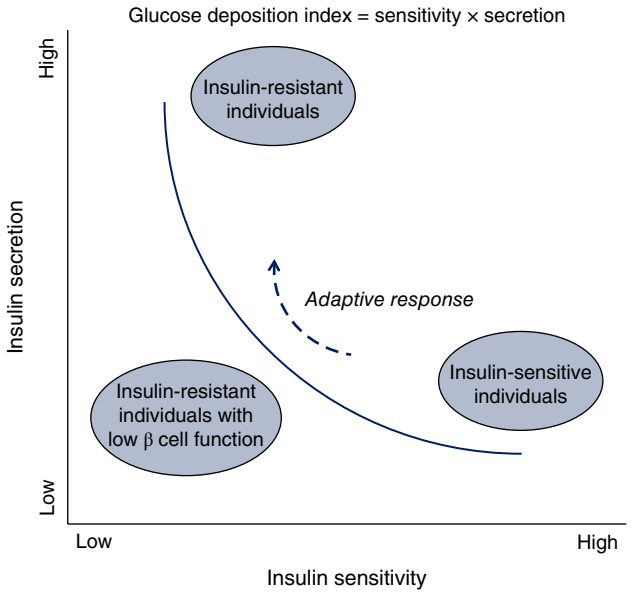

Figure 15.12 Hyperbolic relationship between insulin sensitivity and secretion (Adapted from [395]).

Puberty and ethnicity affect insulin resistance and the risk of progression to diabetes. Puberty imposes a significant degree of insulin resistance and adolescents have been shown to have a 30% lower insulin-mediated glucose disposal when compared with prepubertal children and young adults [399]. This change is transient and there is recovery of insulin sensitivity with completion of puberty. The hormonal mediator for pubertal insulin resistance is thought to be growth hormone. The period is marked by compensatory hyperfunctioning of β-cells, which maintains normal glucose homeostasis. The presence of obesity, ethnicity and genetic susceptibility may tip the scales and lead to dysglycaemia. African American and Hispanic adolescents have a limited capacity of increasing insulin secretion during puberty and therefore a higher risk of progression to T2DM [395].

Defects in β-cell function are an important component of development of impaired glucose tolerance (IGT). Longitudinal follow-up studies of obese adolescents have shown that those who progressed to IGT had impaired β-cell function even at baseline [400]. Recent hyperglycaemic clamp studies in obese adolescents have demonstrated significant impairment in insulin secretion, even in those with normal glucose tolerance [401].

Genetic Factors

T2DM is highly heritable, as evidenced by concordance rates of 50–92% in monozygotic compared to 37–42% in dizygotic twins. Having one parent with diabetes increases the risk of T2DM by 30–40%, and having both parents with diabetes increases the risk by 70%. Common variants have been shown to contribute to 10% risk of T2DM heritability [402, 403]. Genome-wide association studies have increased knowledge of the risk loci associated with T2DM heritability and 75 independent loci have been found to be associated with T2DM [404] (Table 15.11).

Peroxisome Proliferator-Activated Receptor Gamma (PPARG) Gene

Coding variants in *PPARG* gene were one of the first to be found strongly associated with T2DM. PPARG is a transcription factor that plays an important role in adipocyte differentiation. A proline to alanine substitution at position 12 is associated with improved insulin sensitivity and carriers of the more common proline allele are 20% more likely to develop diabetes [406].

Potassium Inwardly Rectifying Channel, Subfamily J, Member 11 (KCNJ11) Gene

A missense polymorphism in the *KCNJ11* gene, which encodes the Kir6.2 ATP-sensitive potassium channel, has been shown to increase T2DM risk with an odds ratio of 1.2. This allele has also been associated with reduced insulin secretion.

Transcription Factor 7-like 2 (TCF7L2) Gene

The most important T2DM susceptibility gene that has been identified is the gene encoding *TCF7L2* and this effect has been replicated in most populations [407]. *TCF7L2* encodes a transcription factor that is a member of the Wnt signalling pathway and known to be active in β-cells. The risk allele is associated with reduced insulin secretion possibly by diminished incretin effect, thereby affecting the enteroinsular axis [408].

Other Genes

Associations have been described with genes causing monogenic diabetes such as *WFS* (leading to Wolfram syndrome) and MODY genes such as hepatocyte nuclear factor *HNF1A*, *HNF1B* and *HNF4A*, but their effect has been moderate. Polymorphisms in the Insulin receptor substrate *IRS-1* and *IRS-2* genes have been associated with increased insulin resistance and risk of T2DM. The melatonin-receptor gene *MTNR1B*, which is related to insulin secretion has also been linked with T2DM risk [402]. Genome-wide association studies have increased the loci associated with T2DM to *KCNQ1*, *SLC30A8* (encoding zinc transporter, ZnT-8), *HHEX*, cyclin-dependent kinase inhibitor 2A/B (*CDKN2A/B*) and *IGF2BP2* genes [405]. Most of these studies demonstrate that susceptibility loci for T2DM are associated with pancreatic development, β-cell function and insulin secretion, indicating that β-cell dysfunction may have a bigger role to play in pathogenesis than insulin resistance (Table 15.12)[403].

Table 15.11 Common genetic loci associated with T2DM risk [405].

Locus	Chr	Risk allele frequency	OR (95%CI)
NOTCH2	1	0.11	1.13 (1.08–1.17)
PROX1	1	0.5	1.07 (1.05–1.09)
IRS1	2	0.61	1.19 (1.13–1.25)
THADA	2	0.92	1.15 (1.10–1.20)
RBMS1/ITGB6	2	0.57	1.11 (1.08–1.16)
BCL11A	2	0.46	1.08 (1.06–1.10)
GCKR	2	0.62	1.06 (1.04–1.08)
IGF2BP2	3	0.29	1.17 (1.10–1.25)
PPARG	3	0.92	1.14 (1.08–1.20)
ADCY5	3	0.78	1.12 (1.09–1.15)
ADAMTS9	3	0.81	1.09 (1.06–1.12)
WFS1	4	0.27	1.13 (1.07–1.18)
ZBED3	5	0.26	1.08 (1.06–1.11)
CDKAL1	6	0.31	1.12 (1.08–1.16)
JAZF1	7	0.52	1.10 (1.07–1.13)
GCK	7	0.2	1.07 (1.05–1.10)
KLF14	7	0.55	1.07 (1.05–1.10)
DGKB/TMEM195	7	0.47	1.06 (1.04–1.08)
SLC30A8	8	0.75	1.12 (1.07–1.16)
TP53INP1	8	0.48	1.06 (1.04–1.09)
CDKN2A/B	9	0.79	1.20 (1.14–1.25)
TLE4	9	0.93	1.11 (1.07–1.15)
TCF7L2	10	0.25	1.37 (1.28–1.47)
HHEX	10	0.56	1.13 (1.08–1.17)
CDC123/CAMK1D	10	0.23	1.11 (1.07–1.14)
KCNQ1	11	0.61	1.40 (1.34–1.47)
KCNJ11/ABCC8	11	0.5	1.15 (1.09–1.21)
CENTD2	11	0.88	1.14 (1.11–1.17)
MTNR1B	11	0.3	1.09 (1.06–1.12)
KCNQ1	11	0.52	1.08 (1.06–1.10)
HMGA2	12	0.1	1.10 (1.07–1.14)
TSPAN8/LGR5	12	0.23	1.09 (1.06–1.12)
OASL/HNF1A	12	0.85	1.07 (1.05–1.10)
PRC1	15	0.22	1.07 (1.05–1.09)
ZFAND6	15	0.56	1.06 (1.04–1.08)
FTO	16	0.45	1.15 (1.09–1.22)
HNF1B	17	0.43	1.12 (1.07–1.18)
DUSP9	X	0.12	1.27 (1.18–1.37)

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Environment and Epigenetic Factors

The worldwide rise in T2DM prevalence underlines the importance of environmental factors since changes in gene pool are unlikely to cause such rapid changes. The increase in T2DM cases, which parallels the obesity epidemic, highlights that rapid environmental changes are leading to increased risk of metabolic diseases.

The thrifty phenotype hypothesis explains that, during the early stages of human evolution, the thrifty genotype originated as adaptive behaviour in times of food scarcity and famine but in today's period of food availability and caloric excess such behaviour has become maladaptive [409]. The fetal origin of adult diseases hypothesis (FOADH) based on Barker's seminal observations states that a critical period of fetal undernutrition may lead to permanent changes in the endocrine metabolic unit that, while favourable in the short time, lead to an increased risk of adult metabolic disease when exposed to a state of postnatal nutrient excess [410]. Fetal reprogramming leads to insulin resistance due to over-activation of the hypothalamic–pituitary–adrenal axis with a change of its set-point leading to increased cortisol concentrations. Pancreatic organogenesis is also affected leading to reduced β-cell mass. The mechanisms leading to increased risk of T2DM due to perinatal reprogramming are summarized in Figure 15.13.

Postnatal environment and caloric status play an important role in modifying risk for development of metabolic syndrome later in life. The match–mismatch theory states that, if the adaptive responses match the postnatal conditions, the phenotype is normal but, if the postnatal condition is one of excess, disease ensues. It has been clearly demonstrated that IUGR children who have rapid catch-up growth and weight gain are prone to develop insulin resistance, PCOS and premature adrenarche in adolescence, and hypertension, diabetes and cardiovascular disease in adulthood [412].

Fetal overnutrition and maternal hyperglycaemia also modulate future risk of metabolic diseases. Infants of mothers with gestational diabetes born large for dates have a higher risk of future development of diabetes than infants who are born with a normal birth weight. Maternal overnutrition also has far reaching consequences, with offspring of women fed high carbohydrate:protein ratio diets having impaired glucose metabolism later in life [411]. The relation between prenatal nutrition and later metabolic disease is probably a U-shaped curve, with increased risk in both underweight and overweight states. Prematurity is an independent risk factor associated with T2DM development which has been shown in the Helsinki birth cohort and other studies [413]. Insulin resistance develops early in life in children who were born premature [414].

Early infant nutrition plays an important role in risk of developing diabetes. Breastfeeding results in a better metabolic profile compared with bottle feeding, which

Table 15.12 Genes predisposing to T2DM.

Gene	Function affected
Reduced insulin secretion	
CDKAL1, CDKN2A, CDKN2B	Reduced beta cell mass
MTNR1B, TCF7L2, KCNJ11	Beta cell dysfunction
Insulin resistance	
FTO	Obesity associated with insulin resistance
IRS1, PPARG	Insulin resistance without obesity

Adapted from [403].

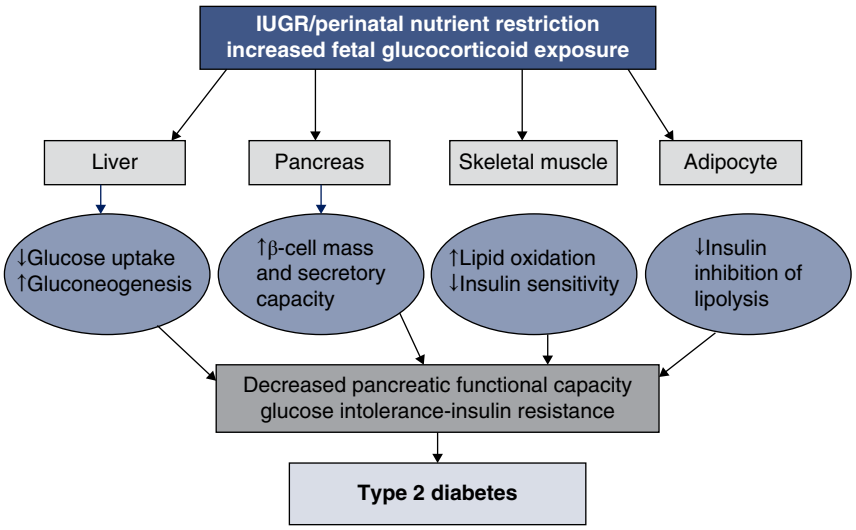

Figure 15.13 The sequence of events leading from intrauterine environment to adult diabetes [411]. *Source:* Reproduced with permission of John Wiley and Sons.

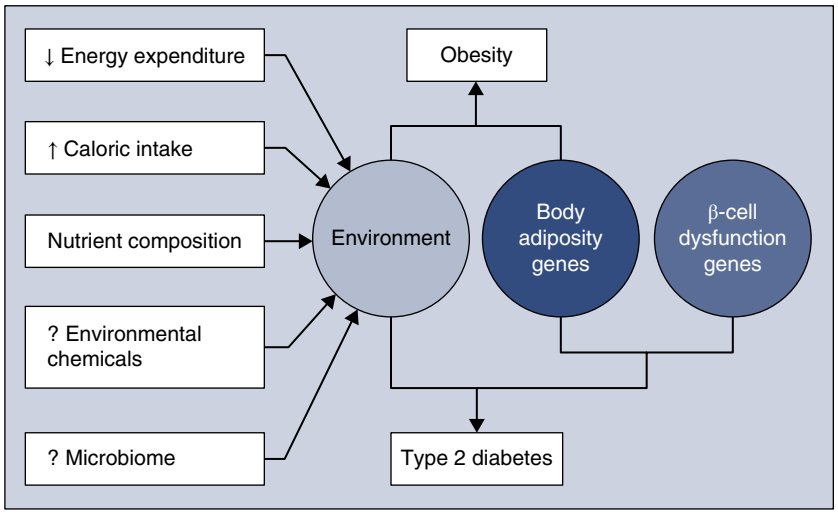

Figure 15.14 Role of genes and the environment in development of obesity and T2DM [422]. *Source:* Reproduced with permission of Springer.

entrains a higher caloric intake and weight gain leading to increased risk of obesity and insulin resistance [412, 415].

Chemicals that act as environmental disruptors have been implicated in the development of T2DM. Possible agents include arsenic, persistent organic pollutants, maternal smoking/nicotine, organotins, phthalates, bisphenol A and pesticides [416]. There have been some data linking environmental exposure to these agents and T2DM risk but these require validation [417].

Recent interest has focused on the role of the intestinal microbiome and its association with obesity, insulin resistance and T2DM [418]. Metagenome-wide association studies have shown that there may be a gut signature in patients with T2DM characterized by a reduction in universal butyrate-producing bacteria and an increase in various opportunistic pathogens [419]. Such differences may act as early biomarkers of T2DM development in at-risk patients [420]. Improvements in insulin sensitivity in patients with metabolic syndrome are seen 6 weeks after infusion of intestinal microbiota from lean individuals [421]. The various possible post-natal influences upon subsequent development of obesity and T2DM are summarized in Figure 15.14.

In addition to the role of genetic variation in the aetiology of T2DM, epigenetics is an important link between environmental changes and nutrition that may alter risk of T2DM disease development. Epigenetics includes processes such as DNA methylation and histone modifications [423]. Additionally, microRNAs are increasingly implicated in the aetiology of conditions such as T2DM. The fetal origin of adult disease hypothesis states that a short-term exposure to an adverse intrauterine environment can affect long-term metabolic disease profiles. Such alterations are unlikely to be due to a genomic change and adverse events that take place during intrauterine life, especially during a specific crucial window of developmental plasticity, may lead to epigenetic modification in various genes, which lead to an adverse metabolic profile in later life [411]. Transgenerational transfer of these metabolic changes has been described.

Further confirmation of epigenetics as a key player in affecting fetal programming of adult disease came from a study that examined methylation profiles in children born before and after maternal bariatric surgery. The offspring born after bariatric surgery were less obese and had a better cardiometabolic profile. The authors reported differential methylation in glucoregulatory genes and genes involved in diabetes-related cardiometabolic pathways [424].

Obesity, Energy Metabolism, Nutrients, Exercise and Epigenetic Modifications

Hypomethylation and differential methylation of *PPAR* α promoters and glucocorticoid receptor have been found, which lead to impaired glucose tolerance and obesity in animal models of protein restriction and global calorie restriction [425]. Intrauterine growth retardation due to placental insufficiency has been associated with progressive epigenetic silencing of Pdx1, impaired ß-cell function and T2DM. Hypomethylation of a CpG site in the fat mass and obesity-associated (*FTO*) gene have been found to be significantly associated with the risk of T2DM in epigenome-wide association studies [426]. Differential methylation at a CpG site in *TXNIP* (a gene associated with skeletal muscle glucose uptake and glucotoxicity-induced β-cell apoptosis) has been associated with T2DM [401]. Obese men and T2DM individuals have also been found to have hypomethylation of genes involved in hepatic glycolysis and insulin resistance [427].

Exercise induces the expression of a number of genes that regulate glucose uptake in skeletal muscle, including glucose transporter isoform 4 (*GLUT4*) by histone acetylation, and enhances transcriptional activity and gene expression. Acute exercise led to promoter hypermethylation and concomitant increase in expression of *PGC-1α*, *PDK4* and *PPAR-δ* (genes associated with skeletal muscle glucose uptake). A 6 month training program also led to differential DNA methylation in adipose tissue genes such as *RALBP1*, *HDAC4* and *NCOR2* that possibly affect adipocyte function [404]. Thus exercise-induced epigenetic modifications may play a role in T2DM susceptibility and disease risk modification.

Preventive Interventions

The obesity epidemic contributes most of the recent T2DM rise in children and adolescents so prevention strategies need to focus on preventing and treating obesity in youth from ethnic minorities. The US Preventive Services Task Force recommends that children 6 years and older be screened for obesity and obese children offered comprehensive moderate- to high-intensity programs that include dietary, physical activity and behavioural counselling components [428]. The cost-effectiveness of behavioural interventions that target diet and physical activity in a school-based environment with a home component has demonstrated childhood obesity prevention [429]. The HEALTHY study was a large intervention based study that evaluated the effects of a 3-year, multicomponent, school-based program on risk factors for T2DM. The primary outcome, the combined prevalence of overweight and obesity, was similar in both the intervention and control schools but the intervention schools did have higher reductions in BMI *z* score, prevalence of obesity and fasting insulin concentrations [430].

Drugs such as sibutramine, orlistat and metformin have been used to treat paediatric obesity. Reductions in BMI with both sibutramine and orlistat have been shown when combined with behaviour intervention but serious side effects limit their use [431]. Metformin has moderate efficacy of weight reduction in severely obese children and adolescents in the short term but no long-term studies are available [432].

In adults metformin reduces risk of progression to T2DM without major side effects by 31%. Acarbose and orlistat have also been shown to reduce the progression to T2DM. Thiazolidinedione anti-diabetic have demonstrated remarkable risk reductions in adult studies but have significant side effects [422]. There are no data on T2DM as a clinical end point in the young but metformin has been shown to improve hyperinsulinemia in obese children with IGT [433].

Diagnosis

The ISPAD 2014 guidelines follow the ADA criteria for diagnosis of diabetes [288, 434]; these are based on the measurement of glycaemia and the presence of symptoms. In the absence of unequivocal symptoms, the society recommends retesting on a subsequent day. Transient hyperglycaemia seen in periods of stress and not associated with symptoms is not diagnostic of diabetes. HbA1C criteria incorporated into adult guidelines have not been validated in pediatric studies and ISPAD recommends caution against relying solely on A1C for diagnosis.

The ADA criteria for testing for T2DM in asymptomatic children are summarized in Table 15.13. Autoantibody testing is recommended for all patients with a clinical diagnosis of T2DM as a high frequency of islet cell autoimmunity has been found in patients with otherwise typical clinically defined T2DM. Autoimmune T2DM is described in cases of clinically diagnosed T2DM with positive autoantibodies but it is best to describe these cases as having autoimmune T1DM presenting in overweight or obese individuals with underlying insulin resistance. Antibody positivity predicts rapid development of insulin dependence [170].

Presenting Features

Obesity is a key feature of T2DM and more than 85% of children with T2DM are overweight or obese. Most children present in the pubertal age group when physiological insulin resistance is at its peak. Family history of T2DM in first-degree family members can be demonstrated in 74–100% of patients. The presentation of T2DM is generally more insidious compared with T1DM. In a third of patients, the diagnosis is made on screening of asymptomatic individuals. Severe presentations are not unknown and up to 25% of children with T2DM may present with DKA. Ketosis and DKA have been demonstrated more commonly in children from

Table 15.13 ADA Criteria for testing for T2DM or prediabetes in asymptomatic children.

Overweight (BMI >85th centile for age and sex, weight for height >85th centile or weight >120% of ideal for height)
Plus 1 or more additional risk factors for diabetes including:
Maternal history of diabetes or gestational diabetes during the child's gestation
Family history of T2DM in first- or second-degree relative
Race/ethnicity (Native American, African-American, Latino, Asian American, Pacific Islander, Southeast Asian)
Signs of insulin resistance or conditions associated with insulin resistance (acanthosis nigricans, hypertension, dyslipidaemia, polycystic ovarian syndrome, small for gestational age)

ethnic minority groups. First presentation with a hyperglycaemic hyperosmolar state (HHS) has been described.

Vaginal candidiasis is a common presenting feature and acanthosis nigricans is seen in the majority of patients. Other co-morbidities such as hypertension, polycystic ovarian syndrome, fatty liver and sleep apnoea can be present at diagnosis in contrast to T1DM where complications occur only years into diagnosis [170, 431, 435, 436].

Differentiating T1DM From T2DM

The increasing incidence in children of T2DM and the current high prevalence of overweight and obesity present clinicians with a diagnostic challenge when evaluating a patient with new-onset DM. Distinguishing T1DM from T2DM may be difficult because of overlap in presentation and some patients have clinical and biochemical features of both types. Ketonuria is unusual in adult patients with T2DM but many adolescents with T2DM have ketonuria or even DKA at presentation. Insulin requirements decrease after several weeks of treatment of T2DM, which may resemble the remission or 'honeymoon' period of T1DM. Measuring pancreatic autoantibodies at the time of diagnosis helps distinguish T1DM from T2DM in obese patients.

Plasma C-peptide concentrations may overlap between the subtypes of diabetes in the acute phase and may not be helpful in classification but persistent elevation of C peptide 12–24 months after diagnosis would be unusual in T1DM [437]. Fasting insulin-like growth factor binding protein-1 (IGFBP-1) level, whose secretion is acutely inhibited by insulin, is a marker of insulinization with a very low IGFBP-1 concentration being suggestive of T2DM [438]. The SEARCH study has suggested a simple algorithm for classification of diabetes based on autoantibody status, insulin sensitivity and waist circumference [439]. The identification of autoantibodies such as GADA, IA-2A, ZnT8 and IAA is highly suggestive of a diagnosis of T1DM. The lack of a positive autoantibody titre is more suggestive of another form of diabetes. In a patient with normal insulin sensitivity who has a normal waist–hip ratio, the differential diagnosis includes MODY, diabetes secondary to other conditions such as cystic fibrosis and other rare forms of diabetes. On the other hand, an increased waist–hip ratio with insulin insensitivity is suggestive of T2DM [439].

Management and Therapies

Consensus and Evidence-Based Guidelines

A number of consensus and evidence based guidelines exist for the care of T2DM in youth. Each year the ADA updates its guidelines in 'Standards of Medical Care in Diabetes', with a specific chapter on Children and Adolescents [337]. The ISPAD issued guidelines in 2018 with a chapter dedicated to T2DM [170]. A joint systematic review and guideline led by the American Academy of Pediatrics, with support from the ADA, the PES, the American Academy of Family Physicians and the Academy of Nutrition and Dietetics addressed management of newly diagnosed patients in 2013 [440]. Diabetes Canada published updated evidence-based guidelines in 2018 [441]. All the documents highlight the lack of evidence, particularly in the area of medical therapies.

Lifestyle, Activity and Diet

Lifestyle intervention is a cornerstone of care for T2DM. Current guidelines recommend at least 60 minutes/day of moderate to vigorous exercise and limiting non-school-related screen time to <2 hours/day [440, 442]. Studies in obese children have shown significant improvement in body composition and cardiovascular risk factors with intensive lifestyle interventions that lead to modest weight loss [443]. Unfortunately, lifestyle interventions added to metformin did not improve glycaemic control when compared to metformin alone [444]. Regular physical activity in youth with T2DM was associated with improved HbA1c and lower BMI [445].

Nutritional approaches have not been well studied in youth with T2DM so recommendations are taken from studies in obese youth and the general population. General principles include reduced calorie consumption to induce gradual weight loss, elimination of sugar-sweetened beverages, increased intake of fruit and vegetables, reduction in processed foods and simple carbohydrates, increase in low glycemic index foods and reduction in portion sizes [442]. Dietary counselling needs to be tailored to the family and, as obesity often involves other family members, should encourage changes in the entire family's lifestyle. Lifestyle changes are difficult to implement so education and counselling should aim to produce incremental improvements in lifestyle choices. Families should be involved in this process and encouraged to provide positive reinforcement for any improvements the child makes. Realistic weight reduction goals should be tailored to each child and be accompanied by counselling to provide the knowledge and motivation to accomplish this difficult task. Attention to mental health issues and psychosocial stresses are key in supporting lifestyle changes [443].

Medical Therapy

Medications

As most youth with T2DM are unable to attain targets for metabolic control with lifestyle changes alone, medication is frequently needed. The only drugs approved for use in children with diabetes in most countries are insulin

and metformin. Insulin is recommended at diagnosis if there is ketosis, random glucose >14 mmol/L or HbA1c >9.0% [442]. Metformin can be added once ketosis has resolved. It is not uncommon for hyperglycaemia to improve in the first weeks after diagnosis, allowing reduction in the dose and even cessation of insulin treatment. Many youth can attain target levels of metabolic control with metformin alone in the post-diagnosis period. The response to metformin is often not lasting and the need for additional therapies is frequent. In the TODAY study of youth with short duration of diabetes, 50% of those treated with metformin had an HbA1c of > 8% over a six month period [444].

Non-Insulin Pharmacotherapies

Oral hypoglycaemic agents produce moderate (1–2%) improvements in HbA1c. Metformin is a biguanide that reduces hepatic glucose production with lesser effects on reduction of intestinal glucose absorption and improvement in glucose uptake and use. It does not improve insulin sensitivity [446] but is the first-line oral hypoglycaemic in youth with T2DM, improving glycaemia without promoting weight gain or hypoglycaemia. A 16-week double-blind randomized clinical trial demonstrated the efficacy and safety of metformin compared with placebo, with an improvement in HbA1c of slightly more than 1% [447]. Metformin has a good safety profile but carries a rare risk of lactic acidosis so is not recommended for those with renal impairment, cardiac and respiratory insufficiency, gastrointestinal illness or receiving radiographic contrast [442]. Common side effects include mild diarrhoea, nausea, dyspepsia, flatulence and abdominal pain, which lessen with time. To minimize side effects, the starting dose is 500 mg/day taken with meals. Dose increments of 500 mg can be made weekly as tolerated, up to a maximum of 2000 mg/day.

Studies of other non-insulin diabetes drugs in those under 18 are limited, despite the large number of drugs approved for use in adults. A sulphonylurea, glimepiride, was as effective as metformin in improving hyperglycaemia in a 26-week randomized comparison study but those treated with glimepiride experienced an increase in BMI Z score compared to a decrease in those treated with metformin [448]. Sulphonylurea drugs are rarely used in youth with T2DM because of this weight gain, hypoglycaemia and poor response due to progressive β-cell failure. They do offer an alternative for patients who cannot tolerate metformin and/or insulin.

In the TODAY study, addition of rosiglitazone to metformin reduced the risk of progression to insulin therapy by 25% when compared to metformin alone [440] but side effects have limited its use. Second line therapy if metformin is not effective in attaining good metabolic control or is not tolerated is insulin, although studies of other drugs such as glucagon-like peptide-1 (GLP-1) receptor agonists, dipeptidyl peptidase 4 (DPP-4) inhibitors and sodium-glucose co-transporter-2 inhibitors (SGLT-2) are in progress. No class of oral hypoglycaemics is recommended above others to add to metformin in adults, with individualized therapy suggested [449]. Table 15.14 describes the most frequently used currently available classes of T2DM drugs based on studies and use in adults.

Insulin Therapy

If optimal metabolic control cannot be attained with lifestyle changes and metformin or in the setting of more significant hyperglycaemia, insulin is required. Addition to metformin of a long-acting basal insulin, such as glargine or detemir at bedtime, starting at 0.25–0.5 units/kg/day, or NPH once daily may be sufficient to bring BG concentrations under control [442, 450] and basal insulin doses are then increased to reach target levels. The addition of a rapid-acting insulin at meal times to achieve target glycaemia is often required as diabetes progresses. There are no studies specifically addressing insulin regimens in T2DM in youth, so the principles outlined for insulin therapy of T1DM have to be used. If adherence is a concern, simple insulin regimens are preferable and premixed insulin preparations may be useful.

Glucose Monitoring

Home glucose monitoring in T2DM is a key component of assessing response to therapy but individualization of the frequency of monitoring is important [442]. Those with good metabolic control managed with lifestyle and/or metformin alone do not require frequent routine monitoring, other than when changes in therapy occur or during intercurrent illness. Those using medications that cause hypoglycaemia such as insulin or insulin secretagogues need more frequent monitoring. If prandial and basal insulin are required, monitoring needs to be increased and practice used for T1DM can be employed.

Bariatric Surgery

Roux-en-Y gastric bypass and sleeve gastrectomy improves many outcomes in adolescents with severe obesity and significant co-morbidities such as T2DM. The largest adolescent study reported remission of T2DM in 95% of obese adolescents at 3 years [451]. Participants lost 27% of body weight; 13% needed further intra-abdominal procedures; 57% had micronutrient and/or vitamin deficiencies.

Models of Care Delivery

High rates of depressed mood and impairment of quality of life are reported in youth with T2DM [452, 453] but, as patients may come from ethnic minority groups with low

Table 15.14 Non-insulin medications used in the treatment of T2DM in adults.

Drug class	Major action	Reduction in HbA1c	Risk of hypoglycaemia	Effect on weight	Other
GLP-1 receptor agonists	Glucose-stimulated insulin release Slowed gastric emptying Reduction of post meal glucagon Reduction of food intake	~1%	Low	Loss of 2–5 kg	Taken subcutaneously daily to weekly Expensive
DPP-4 inhibitors	Prevents breakdown of GIP and GLP-1 with similar action as GLP-1 receptor agonists	~0.5–0.75%	Low	Neutral to small loss	Oral Expensive
Sulphonylureas	Insulin secretagogue	1–2%	Yes	Small gain	Inexpensive
Meglitinides	Insulin secretagogue	Similar to sulphonylureas	Yes	Small gain	Moderately expensive Taken before meals
Thiazolidinediones	Increased insulin sensitivity in fat, liver and muscle	Similar to metformin	Low	Gain 2–5 kg	Moderately expensive Associated with fluid retention, cardiac failure, reduced bone mineral density
SGLT2 inhibitors	Glucose loss in urine	0.5–0.8%	Low	Loss of 2–3 kg	Expensive Side effects of urinary tract infections, vulvovaginal candidiasis, euglycaemic diabetic ketoacidosis

socioeconomic circumstances, it is not surprising that rates of follow-up are poor [454, 455] and care delivery models for T1DM that exist in pediatric diabetes centres need to be adapted for adolescents with T2DM. Successful lifestyle modification programs must be culturally appropriate, family-centred and community based. Recognition of barriers to treatment adherence and clinic attendance need to be assessed and addressed individually.

Therapeutic Goals

The ISPAD recommends a goal of HbA1c of <6.5% for youth with T2DM [442]; the ADA goal is <7.5% [337]. Both recommendations are consensus based.

Acute Complications

Hyperglycaemic Hyperosmolar State and Diabetic Ketoacidosis

While those with T2DM are at lower risk of serious hyperglycaemic events than those with T1DM, both HHS and DKA occur in youth with T2DM. 5–25% of those with new-onset T2DM present in ketoacidosis [294]. DKA in T2DM is defined and treated as in T1DM. Detailed explanations and guidelines for treatment have been published [294]. As some children with DKA also have significant hyperosmolality, there is some overlap between the two syndromes.

Rates of HHS in T2DM are not well described but the risk appears to be higher in T2DM than in T1DM [456]. The criteria for HHS include plasma glucose concentration >33.3 mmol/L, venous pH > 7.25, arterial pH > 7.30, serum bicarbonate >15 mmol/L, mild ketonuria, absent to mild ketonaemia, effective serum osmolality >320 mOsm/kg and altered consciousness (e.g. obtundation, combativeness) or seizures [294]. Cardinal features are severe volume depletion and electrolyte loss. Presenting features tend to be less dramatic than in DKA with increasing polyuria and polydipsia being more subtle. HHS is potentially life-threatening with significant morbidity due to thromboembolic events, rhabdomyolysis and a malignant hyperthermia-like syndrome. Cerebral oedema appears to be rare [456].

Therapy is aimed at restoring volume loss with 20 mL/kg of isotonic saline initially followed by slower correction of volume losses over 48 hours. As ketosis is not significant, insulin is started later than in DKA when the fall in glucose due to rehydration slows and in lower doses, 0.025–0.05 units/kg/hour. Replacement of potassium and phosphate is also important [294]. Use of central venous catheters is associated with increased risk of thrombosis and should be avoided or removed as soon as stable peripheral intravenous access can be obtained. Prophylactic heparin can be used.

Chronic Complications

Co-morbid conditions and complications are common in children with T2DM (Table 15.15). As the condition may have been asymptomatic for many years, screening should begin at diagnosis. Several of the conditions are attributable to insulin resistance rather than to hyperglycaemia and may predate the clinical onset of diabetes. Dyslipidaemia and hypertension should be treated according to current guidelines.

Non-Alcoholic Fatty Liver Disease (NAFLD)

Routine assessment for hepatomegaly and measurement of serum aminotransferase concentrations is required. Elevations of alanine aminotransferase (ALT) are common in children with T2DM, often because of non-alcoholic fatty liver disease (NAFLD), which is characterized by two- to fivefold elevations above the upper limit of normal in ALT and aspartate aminotransferase (AST) concentrations, with ALT greater than AST [459]. NAFLD often has

Table 15.15 Co-morbidities and complications in youth with T2DM diabetes mellitus [442, 457, 458].

Co-morbidity	Prevalence	Screening
Retinopathy (mostly mild non-proliferative)	14% at 5 years 42% at 7 years	Annually from diagnosis by dilated pupil exam or fundus photography
Nephropathy (microalbuminuria)	18–72% within 10 years	Annually from diagnosis by albumin:creatinine ratio in spot urine
Hypertension	10–65%	Routine clinic BP measurement using appropriately sized cuff
PCOS	12–23%	History and physical exam
Dyslipidaemia	4–40%	Lipid profile once diabetes is stabilized after diagnosis
NAFLD	25-50%	Measurement of ALT annually
Sleep apnoea	6%	History of snoring, daytime sleepiness, apnoea
Depression/depressed mood	15–20%	History

NAFLD, non-alcoholic fatty liver disease; PCOS, polycystic ovarian syndrome.

an indolent course but inflammation can progress to chronic liver disease and cirrhosis. Elevated concentrations of AST and ALT typical of NAFLD can be managed conservatively by ruling out other common causes of hepatitis, by promoting gradual weight loss, improving glycaemic control and following ALT concentrations. Mild elevations of ALT are not a contraindication to the use of metformin. More significant elevations of ALT or ALT unresponsive to conservative management should be referred to an appropriate specialist [460].

Polycystic Ovarian Syndrome (PCOS)

Pubertal females should be assessed for menstrual irregularity, amenorrhoea, acne and hirsutism. Lifestyle modification with weight loss improves symptoms. Metformin has been helpful in improving menstrual regularity and reducing hyperandrogenism [461]. Anti-androgens such as spironolactone or flutamide could be considered, although the side effect profile needs to be taken into consideration.

Microvascular Complications

Screening methods and therapy recommendations for microvascular complications are the same as in T1DM. The complication risk is higher than in T1DM of similar duration [462].

Nephropathy

The prevalence of microalbuminuria at diagnosis of T2DM in young persons is 7–22% and increases with diabetes duration. After 5–10 years, macroalbuminuria occurs in 7–17% of patients. These rates are significantly higher than in those with T1DM in the adolescent age range and are associated with higher HbA1c [457]. Patients with T2DM are more likely to have microalbuminuria with lower HbA1c concentrations and shorter duration of diabetes than patients with T1DM. Diagnosis of microalbuminuria requires confirmation of abnormal values in two of three samples taken on separate days. Treatment is with an angiotensin-converting enzyme (ACE) inhibitor [442].

Retinopathy

Mild non-proliferative retinopathy is also found at the time of diagnosis and increases with duration of diabetes and higher HbA1c. In the TODAY study, 14% of patients had early retinopathy after 5 years of diabetes [458]. Screening is by dilated pupil assessment by a trained eye care professional or fundal photography. Guidelines for treatment of retinopathy are the same as for adults.

Neuropathy

Clinically symptomatic neuropathy is very rare in adolescence but screening from the SEARCH study found that 25% of T2DM patients had signs of neuropathy at a mean age of 21.7 years after 7.6 years of diabetes [338].

Macrovascular Complications

The most prominent macrovascular risk factors found at diagnosis are hypertension and dyslipidaemia. Hypertension occurs in ~35% within the first 4 years after diagnosis [463]. Lifestyle intervention is recommended for treatment of hypertension if blood pressure is >90th centile for age, gender and height; an ACE inhibitor should be considered if blood pressure is >90th centile after lifestyle intervention and both lifestyle and ACEI used if blood pressure >95th centile.

Insulin insensitivity is associated with an increased concentration of triglycerides, decreased HDL and small dense LDL particles. 70–80% of youth with T2DM have low HDL and 10–60% have high triglycerides. Lipid goals are: LDL-C < 2.6 mmol/L, HDL-C > 0.9 mmol/L and triglycerides < 1.7 mmol/L. Diet is the first line treatment with the addition of statin therapy if LDL-C remains above 3.4 mmol/L [442]. Arterial stiffness has been found in youth with T2DM, which is suggestive of early atherosclerosis [457].

Prognosis

Metabolic control is often good in the first 2–3 years after diagnosis but frequently deteriorates rapidly. In a study of 331 youth with T2DM from 11 countries in the Western Pacific, the median HbA1c was 7 and 60% achieved a level <7.5% with a mean duration of diabetes of 2.3 years. One-quarter were treated with lifestyle alone, half with an oral hypoglycaemic agent and one-quarter received insulin. More frequent BG monitoring predicted improved outcome. Data from the SEARCH study in the USA showed a mean HbA1C of 7.9% after a mean duration of 2 years [464]. TODAY study findings showed that 45% of participants had an HbA1c > 8% by 3.9 years despite therapy with metformin, metformin and lifestyle intervention or metformin and rosiglitazone and close follow-up [444].

Long-term follow-up of patients is concerning. An Australian study of those diagnosed between 15 and 30 years of age showed that 11% had died at a mean age of 40 with cardiovascular causes of death being most common [462]. A Canadian study showed increased risk of complications (hazard ratio: 1.47) when compared with those with T1DM. Severe complications (dialysis, blindness or amputation) began to appear 10 years after diagnosis [465].

Cystic Fibrosis-Related Diabetes

In many centres the second most common type of diabetes seen is related to cystic fibrosis (CFRD). Management can be challenging for diabetes teams because, apart

from the maintenance of health, many of the imperatives are diametrically opposite to those seen in T1DM. For example, unlike T1DM, CFRD is caused by a non-autoimmune progressive destruction of the endocrine pancreas that affects both α and β-cells. This results in a state of relative, not absolute, insulin deficiency that may be aggravated by an insulin resistance state secondary to chronic inflammation and infection.

Carbohydrate metabolism is further complicated in CFRD by elevated basal energy expenditure, malabsorption, liver disease, glucocorticoid therapy and glucagon insufficiency [466]. In attempting to increase muscle mass and improve lung function in CFRD, insulin is used as much as an anabolic agent as a glucose lowering agent and thus it is desirable to use the maximum possible dose [466]. This contrasts with TIDM management where the lowest effective insulin dose is desirable. Unlike T1DM, high-energy, high-salt and high fat diets are recommended [467].

HbA1C measurements may be spuriously low in CFRD due to high red cell turnover. There is a greater emphasis on post-prandial rather than fasting BG concentrations in CFRD [466]. The main adverse long term outcomes in CFRD are not macrovascular complications; the main causes of morbidity and mortality are lung disease and respiratory failure [467]. Microvascular complications do occur but prevalence is low [466], so, unlike T1DM where good glycaemic control is the primary aim of treatment, the primary goal of therapy in CFRD is the preservation of lung function and optimal nutritional status. HbA1C is measured but is a secondary goal of treatment [466].

Incidence

Survival rates of cystic fibrosis (CF) have improved dramatically over the last few decades to the point where most affected individuals can expect to live into their fourth to fifth decade or longer [463]. As people with CF live longer they experience an increasing risk of CFRD with age-dependent incidences reportedly increasing by 4–9% per year [468]. In screened CF populations, CFRD has been reported with a prevalence of 9% in late childhood, 26% during adolescence and 50% by age 30 [469].

When first described in the 1950s, CFRD was assumed to be caused by obstructive damage to the exocrine pancreas with subsequent enzymatic destruction and fibrosis of the islets within the pancreas. While this process is certainly contributory, it is also nuanced. At autopsy, patients with CFRD show no greater degree of pancreatic fibrosis than CF patients without CFRD [467]. In addition the defective cystic fibrosis transmembrane conductance regulator (*CFTR*) gene is expressed both in α and β-cells. The new 'CFTR-corrector' drug ivacaftor has been shown to improve first-phase insulin response in almost all patients [470].

The risk of developing CFRD is tripled by a family history of type 2 diabetes [471] and increased with genes associated with inflammatory response, tumour necrosis factor, heat shock protein and calpain [472]. These findings indicate a possible dual pathology causing CFRD, a direct molecular defect in β-cells, combined with the indirect impacts of islet destruction and peripheral insulin resistance.

Diagnosis

Consensus statements have emphasized the importance of a proactive approach to screening and diagnosis in an attempt to avoid late, catabolic-driven crisis presentations [473]. The ISPAD and the North American CFRD Guidelines Committee both recommend the use of the oral glucose tolerance test as the mainstay of screening [472, 473]. Fasting BG concentrations and HbA1C are poor predictors of CFRD. The glucose tolerance test is far from a gold standard screening tool with poor reproducibility frequently observed. Since CFRD may be asymptomatic for many years, guidelines recommend annual screening from 10 years of age [472, 473]. An analysis of the baseline, 60 and 120 minute glucose results is combined to determine the four categories of patients (Table 15.16).

Unlike T1DM, patients may move between these categories in positive and negative directions depending on factors such as nutrition, intercurrent health status and medications but, once a diagnosis of CFRD has been made and insulin commenced, it is unwise to cease this unless there is a compelling reason. There should be a low threshold for insulin therapy in a patient who has either indeterminate or impaired glucose tolerance status or unexplained weight loss or deteriorating lung function [472]. The role of continuous glucose monitoring in the diagnosis of CFRD remains unclear.

Management and Therapies

The underlying problem in CFRD is insulin deficiency and guidelines universally recommend insulin replacement [472, 473] but a recent Cochrane review has concluded that there is no evidence that any one insulin regimen or an oral insulin secretagogue (repaglinide) is superior [474]. A variety of insulin regimens has been reported ranging from single daily injections of a long-acting analogue insulin to multiple daily injections to insulin pump therapy. Total daily insulin doses are usually substantially less than those used in patients with T1DM. Therapeutic choices will be determined by individual circumstances (lifestyle issues, gastrostomy

Table 15.16 Results of oral glucose tolerance testing with categories of dysglycaemia [466].

	Baseline glucose (mmol/L)	60 Minute glucose (mmol/L)	120 Minute glucose mmol/L)
Normal	<7.0		<7.8
Indeterminate	<7.0	≥11.1	<7.8
Impaired glucose tolerance	<7.0		7.8–11.1
CFRD	≥7.0		>11.1

Source: Reproduced with permission of Springer Nature.

feeds, etc.). It is not uncommon for young patients with CF to feel overburdened by yet another therapy, particularly a parenteral one. The simplest regimen that achieves the desired outcomes is the most sensible option. The pre-emptive use of insulin to improve weight and lung function in adolescents with CF and impaired or indeterminate glucose tolerance has shown promise [467].

Prognosis

CFRD is associated with an increased risk of mortality, although this risk is declining. Mortality associated with CFRD in the USA has dropped from a 13.4 to a 3.5-fold increased risk from 1992 to 2008 [475]. Cause of death remains pulmonary failure with CFRD exacerbating a decline in lung function through catabolism (loss of muscle mass) and a pro-inflammatory bacteria-permissive environment [472]. Microvascular complications do occur in patients with CFRD duration >10 years. Compared to age, gender and duration of diabetes in T1DM patients, microalbuminuria is more common and retinopathy less common in CFRD patients [466].

Other Dysglycaemia in Cystic Fibrosis

Hypoglycaemia in the absence of treated CFRD has been reported in the context of impaired glucose tolerance, malnutrition, liver disease and gastrointestinal motility disorders. A systematic review was unable to find consistency in circumstance, incidence or definition of this disorder and thus mechanisms and aetiology remain enigmatic [476].

Monogenic Forms of Diabetes

Maturity Onset Diabetes of The Young (MODY)

Maturity onset diabetes of the young (MODY) is a group of disorders characterized by young onset, non-ketotic diabetes that is usually non-insulin requiring and has an autosomal dominant mode of inheritance. It is caused by monogenic defects in β-cell function [477, 478]. The specific β-cell enzymes known to be involved in various MODY subtypes are shown in Figure 15.15. The ADA classifies MODY under the group of 'genetic defects in β-cell function' with a sub-classification according to the gene involved (Table 15.17) [479]. There is significant phenotypic variability of age at presentation, degree of hyperglycaemia, response to treatment, complications and prognosis between the subtypes depending upon the underlying molecular genetic defect [480].

While it has been recommended that the term 'MODY' no longer be used, OMIM continues to describe 14 forms of MODY. The most common causes are mutations in hepatocyte nuclear factor (*HNF)1A* and glucokinase (GCK), and, less commonly, *HNF4A*, *HNF1B* and *INS* [481]. The criteria for a diagnosis of MODY are [482]:

1) Hyperglycaemia usually before age 25 years in at least one and ideally two family members.
2) Autosomal dominant pattern of inheritance, with a vertical transmission of diabetes through at least three generations.
3) Non-insulin dependence or significant C-peptide levels, even in a patient on insulin treatment.
4) Insulin concentrations often in the normal range, although inappropriately low for the degree of hyperglycaemia, suggesting a primary defect in β-cell function.

Overweight or obesity is rarely associated with diabetes in the MODY patients and not required for the development of the condition.

MODY Subtypes (Table 15.17)

GCK *MODY*

The glucokinase enzyme is expressed at high concentrations in the liver and the pancreatic B-cell. It catalyses the rate-limiting step of phosphorylation of glucose to glucose-6-phosphate. Heterozygous inactivating mutations in the *GCK* gene raise the set point for insulin secretion in response to increased BG. Most individuals with heterozygous *GCK* mutations have asymptomatic elevations of fasting plasma glucose to 5.5–8.0 mmol/L [483]. Patients have adequate insulin responses and most have a small increment in plasma glucose 2 hours after

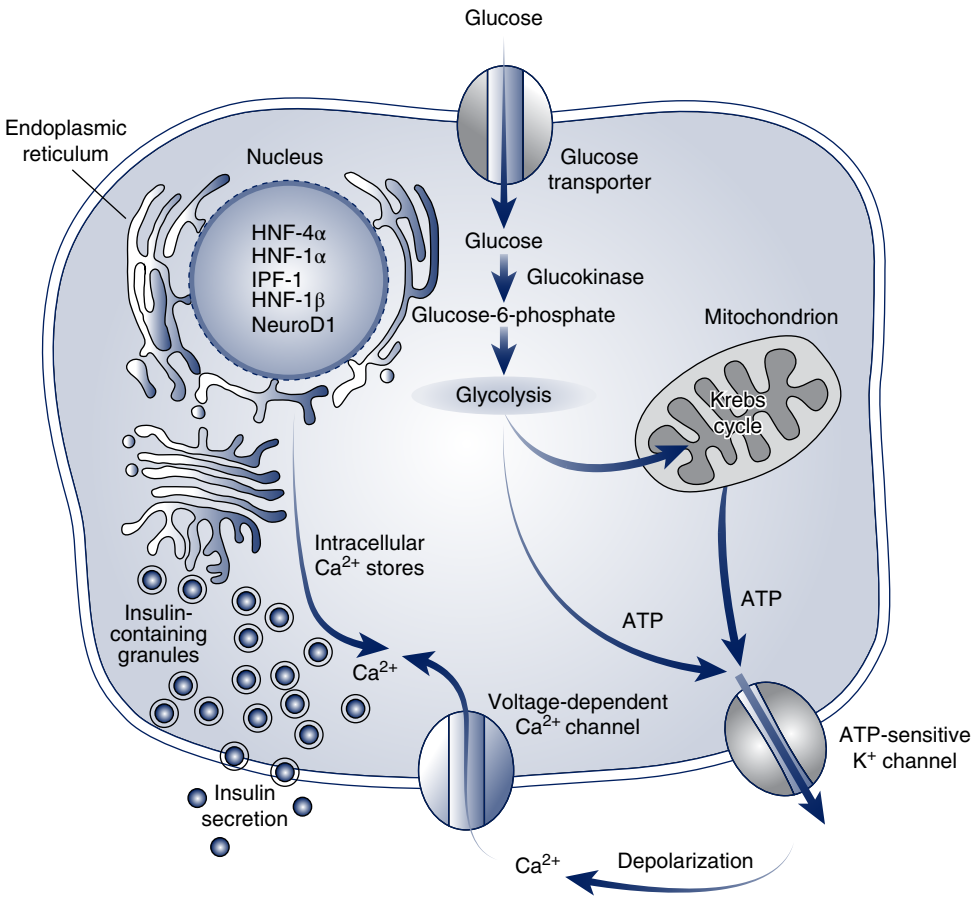

Figure 15.15 Pancreatic β-cell and proteins implicated in MODY pathogenesis [473]. *Source:* Reproduced with permission of John Wiley and Sons. (*See insert for colour representation of the figure.*)

Table 15.17 Classification of maturity onset diabetes of the young (MODY).

Type (MIM)	Gene	Frequency	Associated features	Common treatments
MODY1	*HNF4A*	Uncommon	Glycosuria; reduced serum lipids; macrosomia and hyperinsulinaemic hypoglycaemia in infancy	Sulphonylurea
MODY2	*GCK*	Common	Stable mild hyperglycaemia	Diet and exercise
MODY3	*HNF1A*	Common		Sulphonylurea
MODY4	*IPF-1*	Rare		Oral hypoglycaemic agent, insulin
MODY5	*HNF1B*	Rare	Renal dysplasia, renal cysts	Insulin
MODY6	*NeuroD1*	Rare		Insulin
MODY7	*KLF11*	Rare		Oral hypoglycaemic agent and/or insulin
MODY8	*CEL*	Rare	Pancreatic exocrine dysfunction	Oral hypoglycaemic agent and/or insulin
MODY9	*PAX4*	Rare		Diet, oral hypoglycaemic agent
INS-related[a]	*INS*	Rare		Insulin

[a] As yet unnamed.

an oral glucose load (<3 mmol/L in 70% of patients) [484]. HbA1C values are rarely >7.5% [485] and micro- or macrovascular complications are rare [486]. Most children are asymptomatic and detected upon the finding of incidental hyperglycaemia [487]. In most cases, the affected parent is undiagnosed or has been diagnosed to have early onset T2DM.

Generally GCK MODY does not require treatment and glucose lowering measures have no effect on glycaemia. *GCK* gene mutations can lead to gestational diabetes with implications on fetal growth. If a baby does not inherit a *GCK* mutation from its affected mother, it will be at risk of macrosomia [488, 489]. Insulin treatment may be required in pregnancy if fetal macrosomia is detected.

HNF1A *and* HNF4A *MODY*

The *HNF1A* and *HNF4A* genes encode transcription factors important for pancreatic development and β-cell differentiation and function. Diabetes occurs with mutations of either gene due to progressive β-cell dysfunction. Most cases present before the age of 25 years and have a large increment of glucose in an OGTT (>5 mmol/L). *HNF1A* mutation carriers can have normal fasting glucose levels. The risk of developing long term micro- and macrovascular complications is similar to that in T1DM or T2DM and is related to long term glycaemic control. Both these types of MODY respond to sulphonylurea medication [486].

In a large UK series, mutations in the *HNF1A* gene were shown to be the commonest cause of MODY [481]. The condition has high penetrance, with 63% of carriers developing diabetes by 25 years of age, 79% by 35 years and 96% by 55 years [486]. Carriers have a low renal threshold for glucose and develop glycosuria before clinical diabetes [490]. They have a higher than normal HDL concentration in contrast to the low concentration normally observed in T2DM and the normal concentrations seen in T1DM [486]. Apolipoprotein M concentrations are low in HNF1A MODY and a useful marker to differentiate it from T1DM [491]. High sensitivity C-reactive protein (hsCRp) concentrations are significantly lower in HNF1A cases than other forms of diabetes and may be a useful screening test [492].

Mutations in *HNF4A* account for about 10% of MODY cases [478–481]. The clinical features are very similar to HNF1A MODY and most cases are detected by the age of 25, although some families may be detected at older ages. Fetal macrosomia and diazoxide-responsive neonatal hyperinsulinaemic hypoglycaemia has been described in heterozygous carriers of the *HNF4A* mutation [493, 494].

Most patients with HNF1A and HNF4A MODY are very sensitive to sulphonylureas [495] and these are recommended as first-line treatment [496], which can be maintained in small doses for prolonged periods of time. Meglitinide has been studied in adolescents with HNF1A MODY and produces lower rates of hypoglycaemia with similar glycaemic control [497]. GLP1 agonists have lower rates of hypoglycaemia than sulphonylureas [498].

HNF1B (Renal Cysts and Diabetes Syndrome)

HNF1B is a transcription factor expressed in the pancreas, kidneys, liver, genital tract and gut and mutations cause monogenic diabetes with predominantly extrapancreatic features. Mutations rarely cause MODY [481] and the commonest phenotype is developmental renal disease. Renal cysts are most commonly detected but renal dysplasia, renal tract malformations and/or familial hypoplastic glomerulocystic kidney disease may occur [498]. Diabetes is seen in about half of the cases, generally by adolescence [500]. Genital tract malformations (particularly uterine abnormalities), hyperuricaemia, mental retardation and gout can occur, as well as abnormal liver function tests [496]. Hypoplasia of the pancreas and exocrine pancreatic insufficiency have been described as part of the spectrum of *HNF1B* gene disorders [501]. Patients have insulin resistance and do not respond adequately to sulphonylureas requiring early insulin therapy [496].

Differentiating MODY From Other Diabetes Subtypes

MODY accounts for 1–2% of all diabetes cases [486] but a large proportion of MODY probably remains undiagnosed or misclassified as T1 or T2 DM [481, 486]. Distinguishing MODY from T1DM and T2DM can be challenging and should be considered in people with atypical features (Figure 15.16).

Guidelines suggest testing for MODY in a patient with apparent T1DM when there is (1) a family history of diabetes in one parent or first degree relatives, (2) absence of islet autoantibodies and (3) low or no insulin requirements 5 years after diagnosis [496]. Using these criteria, a 50% detection rate of MODY was found in children with multiple atypical features offered genetic screening for *GCK* and *HNF1A* genes [503].

Distinguishing MODY from T2DM can be more difficult because of the high likelihood of having a positive family history in T2DM. Absence of obesity, lack of features of insulin resistance, such as acanthosis nigricans or metabolic syndrome, and an ethnic background with a low prevalence of T2DM, (e.g. European Caucasians) should prompt a search for monogenic diabetes [502]. Observational data from the DPV-Wiss database from Germany and Austria found that children with MODY were younger at diagnosis and had a lower BMI than those with T2DM. It was also found that, while macrovascular risk factors such as dyslipidaemia and

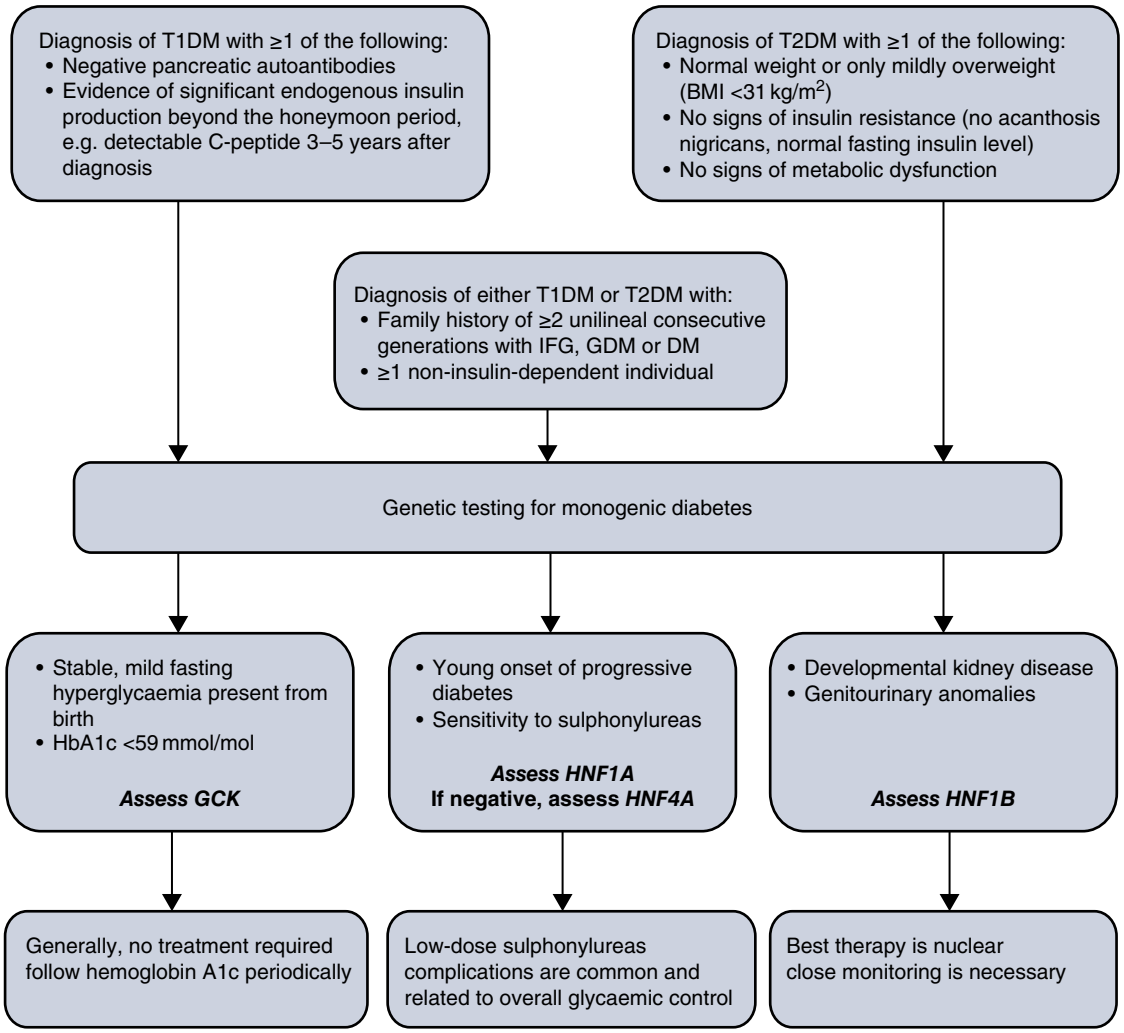

Figure 15.16 Suggested approach to MODY genetic testing [502]. *Source:* Reproduced with permission of John Wiley and Sons.

hypertension were more common in T2DM, they also occurred in a significant proportion of those with MODY [503]. The risk of developing long term micro- and macrovascular complications is similar to that in T1DM and is related to long term glycaemic control.

Neonatal Diabetes Mellitus (NDM)

NDM is a rare form of diabetes presenting very early in life which can be attributed to genetic defects in insulin secretion or synthesis in most cases. Traditionally it was defined as the onset of hyperglycaemia within the first month of life, hence the name neonatal diabetes, but the age limit has progressively increased to 6 months (with some reports of onset up to 9–12 months of age), as it becomes apparent that autoimmune diabetes is rare in this age group. The exception to this rule is immune dysregulation, polyendocrinopathy, enteropathy and X-linked (IPEX) syndrome, which is caused by mutations in the *FOXP3* gene resulting in a loss of T regulatory cell function and leading to NDM, which is autoimmune in nature [496].

Some have considered the term NDM to be a misnomer and have suggested the alternative term of monogenic diabetes of infancy [505]. Neonatal diabetes remains the term in most common usage. It can be classified into transient (TNDM) seen in up to half the cases, which remits within a few weeks or months of life, and permanent (PNDM), which requires life-long treatment [506].

Neonatal diabetes is said to occur in 1:300,000–400,000 newborns. Of all cases with neonatal diabetes, 45% have

a transient form, 45% have a permanent form and 10% are syndromic or have pancreatic aplasia [507]. The SEARCH study found that, of the 15,829 participants with diabetes, 39 were diagnosed before 6 months of age. Of these 39, 35 had PNDM and 3 had TNDM, which had remitted by 18 months of age. The population prevalence of NDM was estimated to be 1 in 252,000 < 20 years of age [508].

Most children are born with intrauterine growth retardation due to severe insulin deficiency. Presentation can range from failure to thrive and dehydration to life threatening ketoacidosis and coma [509]. Most children have isolated diabetes but some have associated extra pancreatic features, which can often give a clue to the genetic aetiology and aid in diagnostic testing [505].

Transient Neonatal Diabetes Mellitus (TNDM)

70% of cases of TNDM can be attributed to overexpression of the paternally expressed genes *ZAC* and *HYMAI* due to imprinting abnormalities in the 6q24 region. The molecular mechanisms underlying this anomaly are paternal duplication, paternal uniparental disomy and abnormal methylation of the maternal allele. Uniparental disomy is usually sporadic and accounts for 50% of cases; in cases of paternal duplication, fathers have a 50% risk of passing on the disease to their children.

Some cases of TNDM secondary to multiple methylation defects are caused by mutations in the *ZFP57* gene involved in the regulation of DNA methylation on chromosome 6p and these are inherited in a recessive manner [496]. The molecular mechanisms of 6q24 associated NDM are unclear but the *ZAC* gene regulates cell cycle arrest and apoptosis and therefore overexpression can lead to reduction of fetal β-cell mass and IUGR secondary to reduced insulin secretion.

The function of HYMAI is not well known [506]. Affected children typically present in the first few days to weeks of life with IUGR and non-ketotic hyperglycaemia.

Activating mutations in *KCNJ11* (11%) and *ABCC8* (15%) genes account for the remaining cases of TNDM. Mutations causing TNDM have a less pronounced effect on channel function compared with mutations that cause PNDM. The ability of ATP to close the channel *in vitro* correlates with the severity of neonatal diabetes mellitus. In a study of 97 TNDM cases, it was noted that neonates with 6q24 anomalies had a lower average birth weight and were diagnosed and remitted earlier compared with those with KATP channel mutations [510].

Permanent Neonatal Diabetes Mellitus (PNDM)

PNDM Due to Mutations in the KATP Channel Genes

The KATP channel is an octameric complex made up of four Kir 6.2 (ATP-sensitive inward rectifier potassium channel) and four SUR1 (sulphonylurea receptor 1) subunits. Following a carbohydrate meal, glucose is transported into the pancreatic β cell by the GLUT2 transporter. Glucokinase (GCK) then converts glucose to glucose-6-phosphate, which is subsequently metabolized by the glycolytic and Krebs cycle pathway to produce ATP (Figure 15.17). An increase in the intracellular ATP/ADP ratio leads to closure of the K ATP channels and depolarization of the β cell membrane, which allows influx of Ca^{2+} across the voltage gated Ca^{2+} channels and exocytosis of insulin containing granules [506].

Activating mutations in the *KCNJ11* gene (31% of PNDM), which encodes Kir 6.2, and the *ABCC8* (13% of PNDM) gene that encodes SUR1 prevent channel closure in response to ATP and therefore lead to impaired insulin secretion. *KCNJ11* mutations are found more frequently in PNDM and are usually *de novo* in 90% of cases, with autosomal dominant inheritance in the others. *ABCC8* mutations cause TNDM more often and mutations in this gene can be inherited in a dominant, recessive or a compound heterozygous manner [496].

The Kir6.2 channel in also expressed in neurons, brain and muscle and about 20% of patients with *KCNJ11* mutations present with neurological involvement. The most severe form of this condition is called developmental delay, epilepsy and neonatal diabetes (DEND) syndrome. A less severe form is intermediate DEND (iDEND) where less severe developmental delay is seen without epilepsy. Neurological features have also been seen to be associated with *ABCC8* mutations but they are less common and usually milder (language delay and dyspraxia) [512]. A milder degree of neuropsychological dysfunction, such as visual-spatial dyspraxia or attention deficits, may be found on detailed testing in all patients with KATP channel mutations [513].

PNDM Due to Mutations in *INS* Gene

Dominant mutations in the *INS* gene are the second commonest cause of PNDM (16%). The *INS* gene encodes for the preproinsulin molecule and mutations result in misfolding of the molecule. The abnormal molecule is accumulated in the endoplasmic reticulum leading to ER stress and β cell apoptosis due to the unfolded protein response. Most children have IUGR due to *in utero* insulin deficiency. Diabetes usually presents later than patients with KATP channel mutations and requires treatment with insulin. Heterozygous mutations are de novo in 80% of cases. Homozygous and compound heterozygous modes

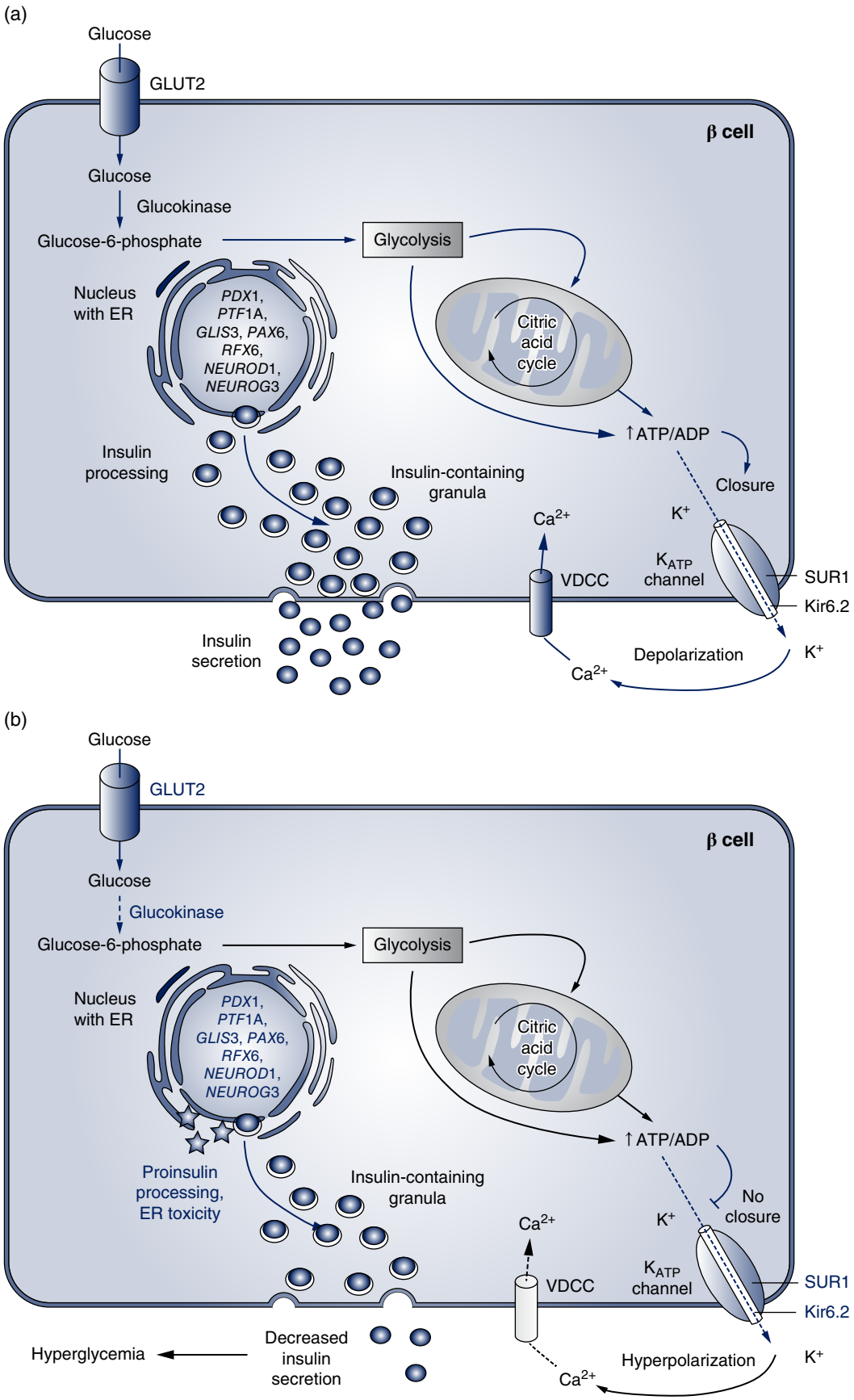

Figure 15.17 Pathogenesis of decreased insulin secretion in neonatal diabetes mellitus. (a) Normal intracellular response to influx of glucose. (b) Impaired cellular response to glucose and implicated genes in blue [511]. *Source:* Reproduced with permission of Springer.

of inheritance are also seen. In the homozygous forms of the disease, the phenotype is much more severe due to reduced insulin biosynthesis and children present earlier and with a more severe degree of IUGR [496].

INS gene mutations also present after the age of 6 months and can be difficult to distinguish from T1DM since both are insulin requiring. Family history may be lacking because most INS mutations are *de novo*. Pancreatic autoantibody testing in young children with diabetes may help identify candidates for genetic testing [505].

PNDM Due to *GCK* Mutations

Homozygous or compound heterozygous mutations in the *GCK* gene causing complete glucokinase deficiency can lead to PNDM due to impaired insulin secretion from the β cell in response to hyperglycaemia (3% of PNDM). As there is insulin deficiency *in utero* most children are born with severe IUGR. Due to failure of postnatal insulin secretion most children are diagnosed early and require exogenous insulin. As this type of PNDM is inherited in a recessive manner, it should be suspected in consanguineous families. Testing fasting BG in an asymptomatic parent can help in making the diagnosis [496].

Syndromic Neonatal Diabetes Mellitus

These account for 10% of neonatal diabetes. In 45% of cases, a molecular basis has been identified (e.g. *PTF1A*, *FOXP3*, *EIF2AK3*, *HNF1B*, *IPF1*).

Wolcott-Rallison Syndrome (WRS)

WRS is caused by mutations in the *EIF2AK3* gene encoding eukaryotic translation initiation factor 2-α kinase 3 and inherited in a recessive manner. Typical features include NDM and spondyloepiphyseal dysplasia as well as recurrent hepatic and/or renal dysfunction, cognitive dysfunction, hypothyroidism, pancreatic exocrine insufficiency and neutropenia with recurrent infections [514]. The EIF2AK3 protein is important in the endoplasmic reticulum stress response and mutations in the protein lead to accumulation of malfolded proteins in the ER leading to ER dysfunction and β-cell apoptosis. Neonatal diabetes is the first manifestation and develops in early infancy but later onset has also been described with the epiphyseal dysplasia becoming apparent within the first 1–2 years of life. WRS is the commonest genetic cause of PNDM in consanguineous families [515].

Immune Dysregulation, Polyendocrinopathy, Enteropathy and X-Linked (IPEX) Syndrome

IPEX syndrome is an X-linked disease caused by mutations in the *FOX P3* gene, a transcriptional regulator for CD4 regulatory T cells. Mutations lead to Treg cell dysfunction, which results in multiorgan autoimmunity. Male infants present with early onset diabetes, eczema and enteropathy. The diabetes is autoimmune and associated with β-cell autoantibodies. Enteropathy presents as intractable diarrhoea with villous atrophy. Many infants succumb to life-threatening infections as a result of immune deficiency. Later manifestations can include primary hypothyroidism, nephritis, hepatitis, enteritis and alopecia. Immunosuppressive agents such as sirolimus and steroids are used for treatment. Bone marrow transplant offers a potential cure [481, 496, 512].

Other causes of neonatal diabetes are described in Table 15.18. Most are due to mutations in transcription factors involved in pancreatic development. When a transcription factor that is expressed early in development is affected, it leads to both endocrine and exocrine components being affected whereas, if a transcription factor specific to the endocrine component is affected, the exocrine function of the pancreas is normal. Functional tests of exocrine pancreatic function (e.g. faecal elastase and faecal fat) may need to be tested to assess pancreatic hypoplasia or aplasia; imaging of the pancreas may be unreliable in infancy [496, 503].

Diagnosis

Searching for a genetic diagnosis in children with neonatal diabetes is highly desirable as it has important implications for treatment, prognosis and risk of disease recurrence in siblings. A molecular diagnosis should be sought in a child with onset of diabetes at <6 months of life. Testing for KATP channel mutations has been shown to be cost-effective [516].

Sequential testing for *KCNJ11*, *ABCC8*, *INS* and 6q24 abnormalities will identify most cases of NDM. If these genes are found to be normal, more targeted testing may be needed. A detailed history and examination can help guide the testing. A family history of consanguinity suggests an autosomal recessive inheritance. A history of mild diabetes or asymptomatic hyperglycaemia can be found in *GCK* mutations. Male infants with neonatal diabetes who are found to have autoantibodies should be tested for IPEX syndrome. The presence of extra-pancreatic features can also point to specific gene mutations such as *HNF1β*, *GATA6*, *EIF2AK3* and *WFS1* [505].

Next-generation sequencing may help in simultaneous analysis of multiple genes at a low cost and will possibly be the test of choice in the future.

A suggested approach to traditional genetic testing is suggested in Figure 15.18.

Table 15.18 Monogenic subtypes of neonatal and infancy-onset diabetes mellitus.

Gene	Locus	Inheritance	Other clinical features
Genes leading to abnormal pancreatic development			
PLAGL1	6q24	Variable (imprinting)	TNDM, macroglossia, umbilical hernia
ZFP57	6p22.1	Recessive	TNDM (multiple hypomethylation syndrome), macroglossia, developmental delay, umbilical defects, congenital heart disease
PDX1	13q12.1	Recessive	PNDM, pancreatic agenesis
PTF1A	10p12.3	Recessive	PNDM, pancreatic agenesis, cerebellar hypoplasia/aplasia, central respiratory dysfunction
HN1FB	17cen-q21.3	Dominant	TNDM, pancreatic hypoplasia, renal cysts
RFX6	6q22.1	Recessive	PNDM, intestinal atresia, gall bladder agenesis
GATA6	18q11.1-q11.2	Dominant	PNDM, congenital heart defects, biliary abnormalities
GLIS3	9p24.3-23	Recessive	PNDM, congenital hypothyroidism, glaucoma, hepatic fibrosis, renal cysts
NEUROG3	10q21.3	Recessive	Enteric anendocrinosis
NEUROD1	2q32	Recessive	PNDM, cerebellar hypoplasia, visual impairment, deafness
PAX6	11p13	Recessive	PNDM, microphthalmia, brain malformation
Genes associated with abnormal β-cell function			
KCNJ11	11p15.1	*De novo*/dominant	PNDM/TNDM, DEND
ABCC8	11p15.1	*De novo*/dominant/ recessive	PNDM/TNDM, DEND
INS	11p15.1	Dominant/recessive	Isolated TNDM/PNDM
GCK	7p15-p13	Dominant/recessive	Isolated PNDM
SLC2A2 (GLUT2)	3q26.1-26.3	Recessive	Fanconi-Bickel syndrome, PNDM, hypergalactosaemia, liver dysfunction
SLC19A2	1q23.3	Recessive	PNDM, thiamine-responsive megaloblastic anaemia, sensorineural deafness
Genes associated with destruction of β-cells			
INS	11p15.1	*De novo*/dominant	Isolated PNDM
EIF2AK3	2p12	Recessive	Wolcott-Rallison syndrome, PNDM, skeletal dysplasia, recurrent liver dysfunction
IER3IP1	18q12	Recessive	PNDM, microcephaly, lissencephaly, epileptic encephalopathy
FOXP3	Xp11.23-p13.3	X-linked, recessive	IPEX syndrome (autoimmune enteropathy, eczema, autoimmune hypothyroidism, elevated IgE)
WFS1	4p16.1	Recessive	PNDM, optic atrophy, diabetes insipidus, deafness

Adapted from [505].

Treatment

Most children with neonatal diabetes need insulin therapy to prevent acute decompensation and allow normal growth and development pending a molecular diagnosis. Insulin can be given by injections or by pump. Small doses are generally used to start and dilution of insulin may be necessary. With increasing oral feeds, meal time boluses are needed [505, 511]. More than 90% of cases with KATP channel gene mutations can be transferred from insulin onto sulphonylurea medications. Binding of sulphonylurea to SUR1 induces ATP-independent channel closure in response to high glucose resulting in insulin secretion [517]. Patients treated with sulphonylureas have been shown to have meal-stimulated insulin secretion with reduced risk of hypoglycaemia as compared to insulin therapy [512]. The sulphonylurea dose per kilogram required is much higher than that needed by adults with T2DM and 0.5 mg/kg is a usual starting dose [505].

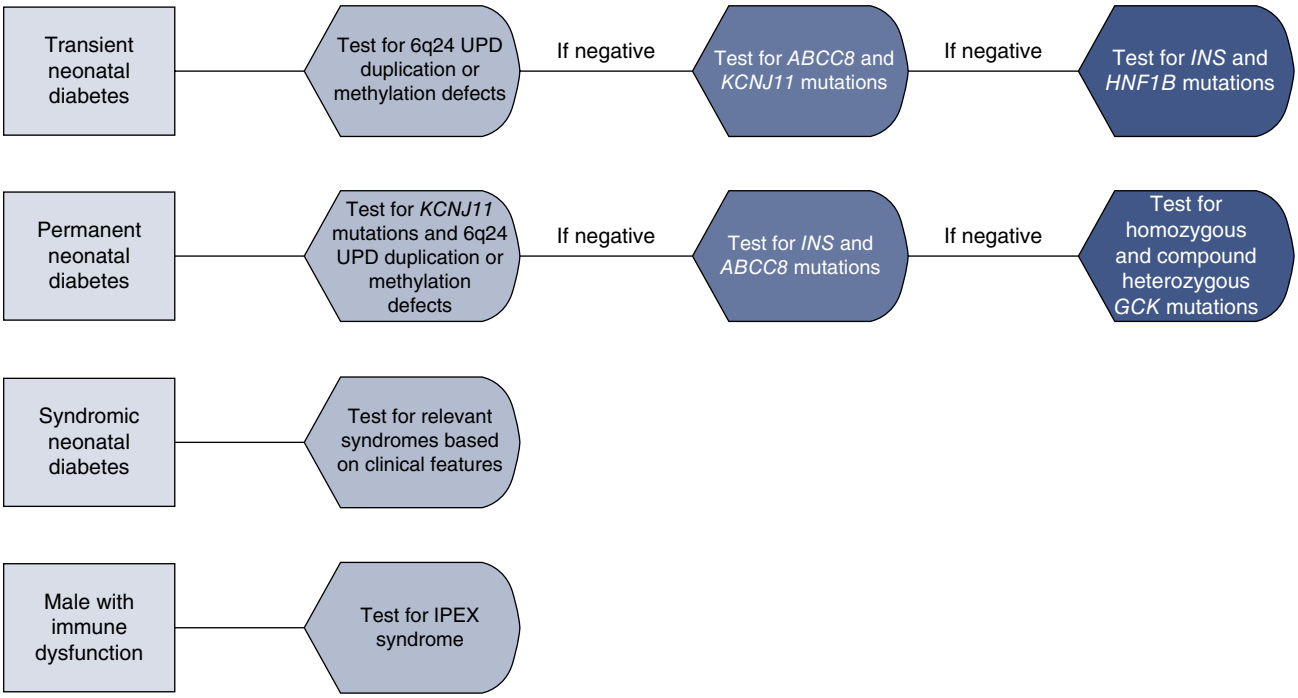

Figure 15.18 Approach to genetic testing for neonatal diabetes. If it is unclear if diabetes is permanent or transient, testing for both KCNJ11, the most common cause of permanent neonatal diabetes, and 6q24 chromosome abnormalities, the most common cause of transient neonatal diabetes, should be pursued [512]. *Source:* Reproduced with permission of John Wiley and Sons.

Transfer protocols are available at http://www.diabetes-genes.org/. Patients with iDEND syndrome have shown improvement of neurological features on sulphonylurea therapy [518].

Rare Forms of Diabetes

Defects in Insulin Secretion

Mitochondrial Diabetes

Diabetes may be the presenting manifestation of syndromes caused by mutations in mitochondrial DNA. Maternally inherited diabetes and deafness (MIDD) syndrome may present in children. The most common mutation occurs at position 3243 in the tRNA leucine gene, leading to an A-to-G transition [519]. This and other mutations in related tRNA mitochondrial genes can also be associated with many other features including myopathy, encephalopathy, lactic acidosis and myoclonic epilepsy. Kearns-Sayre syndrome, also caused by mitochondrial gene mutations, is characterized by ophthalmoplegia, retinal pigmentary degeneration and cardiomyopathy; it may include several hormone deficiencies including diabetes in ~13% of cases. Diabetes can be treated with diet and sulphonylureas but may require insulin. Patients with impaired mitochondrial function are prone to develop lactic acidosis and therefore metformin should not be used [520].

Other Molecular Disorders

Thiamine-responsive megaloblastic anaemia syndrome is caused by mutations in a thiamine transport gene (*SLC19A2*) and is often accompanied by diabetes mellitus and/or sensorineural deafness. Treatment with thiamine corrects the anaemia and sometimes improves the diabetes but insulin is often required [521]. Rare defects in prohormone convertase activity inherited in an autosomal dominant pattern lead to impaired processing of proinsulin and mild glucose intolerance. A few families have been identified who secrete mutant insulins with impaired ability to bind to the insulin receptor. Glucose metabolism may be normal or only mildly impaired in these individuals.

Impaired Insulin Sensitivity

Genetic Defects of Insulin Signalling

Several rare insulin resistance syndromes are caused by genetic defects in the insulin receptor or its cellular signalling apparatus.

Leprechaunism (Donohue syndrome) is the most severe, presenting at birth with low birthweight, characteristic facial features, near-total lack of adipose tissue, acanthosis nigricans and extreme insulin resistance. It is

usually fatal in infancy, although there are some case reports of treatment with IGF-1 [522].

Rabson–Mendenhall syndrome is characterized by extreme insulin resistance with acanthosis nigricans; abnormalities of the skeleton, teeth and nails; growth retardation; genitomegaly; and pineal gland hyperplasia.

Type A insulin resistance syndrome typically presents in thin young women with extreme hyperinsulinism, acanthosis nigricans, glycosuria, hyperandrogenism with virilization and PCOS [523].

Inherited Lipoatrophic Diabetes

Lipoatrophic diabetes is associated with widespread loss of adipose tissue and severe insulin resistance. Hyperlipidaemia, hepatomegaly, acanthosis nigricans and elevated basal metabolic rate are common. Several forms of the disease are caused by gene defects: Seip–Berardinelli syndrome is inherited as an autosomal recessive and usually presents in the first year of life with lack of subcutaneous adipose tissue. Insulin resistance, acanthosis nigricans and diabetes mellitus develop before adolescence; familial partial lipodystrophy (Dunnigan syndrome) caused by an autosomal dominant mutation in the lamin A/C gene or peroxisome proliferator-activated receptor gamma gene presents in adolescence with loss of subcutaneous adipose tissue from the trunk and extremities but with excess adipose tissue on the face and neck.

Acquired Insulin Resistance

Severe generalized acquired lipoatrophy may present during childhood. Diabetes ensues within a few years of the loss of adipose tissue. Some forms of acquired lipoatrophic diabetes are caused by immune-mediated destruction of adipocytes and are often associated with other autoimmune diseases. Some patients with HIV disease treated with protease inhibitors develop partial lipodystrophy. Type B insulin resistance syndrome resulting from circulating antibodies directed against the insulin receptor is a rare cause of diabetes [524].

Diabetes as a Component of Specific Genetic Syndromes

In Wolfram syndrome (DIDMOAD, diabetes insipidus, diabetes mellitus, optic atrophy and deafness), insulin-deficient diabetes mellitus is often the presenting feature at a median age of 6 years [525]. Most cases have an identifiable mutation of the Wolframin gene inherited in an autosomal recessive fashion. Other syndromes that are associated with an increased risk of diabetes include Alstrom, Prader–Willi and Bardet–Biedl syndromes that combine severe obesity with insulin-resistant diabetes mellitus.

Living with Diabetes

The goals of management of diabetes in children and adolescents are to allow them to lead as normal a life as possible while maintaining metabolic control to avoid short- and long-term complications. Good control is associated with better quality of life [254] but patients and their families require comprehensive diabetes education to provide them with the skills and knowledge to manage the disease on a daily basis. Ongoing education should address transitions such as starting school, adolescence and entering adult diabetes care. Diabetes presents a heavy burden on the child and family and health professionals need to be vigilant for signs of burn out and depressed mood. Support can be gained from diabetes team members, mental health professionals, families of children with diabetes, peers, support groups, online communities, diabetes camps and associations.

Education

As children spend a significant portion of their weekdays at school, diabetes care needs to be supported in school. Parents need clear plans with reasonable expectations for schools and training school personnel that include [253]:

- Awareness of factors that affect glucose levels, such as food intake and physical activity.
- Communication plan with parents.
- BG testing until children are capable of testing independently.
- Identification and treatment of hypoglycaemia, including how to treat severe hypoglycaemia with glucagon if possible and when to call emergency services.
- Plans for food or insulin adjustment for activity.
- Ensuring access to meals and snacks and treatment for hypoglycaemia.
- Insulin dose verification and where possible administration by injection or as a bolus with an insulin pump.
- Accommodation during examinations if hypoglycaemia occurs.

Many national diabetes organizations have published guidelines for school care that help support children at school.

Employment

Many adolescents begin part-time and summer employment and face challenges in explaining their condition to supervisors and colleagues, feeling uncomfortable doing so. They may also feel self-conscious about glucose testing, insulin administration and treatment of hypoglycaemia. Diabetes health professionals can provide support.

Many countries provide protection for employees with health conditions from discrimination and have rules/laws that accommodate healthcare needs. Patients and their families can find detailed information about rights of employees with diabetes from their national diabetes organizations. In the past, those with diabetes were considered to be ineligible for certain types of employment but recent efforts by many advocacy groups has led to the recognition that those with diabetes should be considered for employment based on the requirements of the position and the individual's medical condition, treatment regimen and medical history [526]. Studies have found slightly lower rates of employment in those with childhood onset diabetes during adult life but the effects were due to the development of diabetes complications. Career attainment is similar to siblings as long as health issues do not interfere [527].

Driving

Most drivers who have diabetes do not experience difficulties but hypoglycaemia due to treatment with insulin or insulin secretagogues is a constant hazard. Driving ability begins to be impaired when BG is <3.8 mmol/L and drivers may not be aware of the impairment [528]. Slightly higher rates of accidents in those with diabetes have been reported with higher risk in those with T1DM, hypoglycaemia unawareness, history of severe hypoglycaemia and less frequent testing of BG before driving [528]. Recommendations for driving have been developed in many countries:

- Always carry a glucose meter and BG test strips.
- Check BG before driving and every 2–4 hours while driving.
- If BG is 4.0–5.0 mmol/L, take a snack before driving.
- If glucose is <4.0 mmol/L or the driver has symptoms of hypoglycaemia, do not drive. Wait 45 minutes after recovery of BG before driving.
- If hypoglycaemia develops while driving, stop the vehicle as soon as possible.
- Always keep a supply of fast-acting carbohydrate within easy reach in the vehicle.

Patients should be educated about these recommendations as soon as they have reached the age of eligibility for a driver's licence. Many jurisdictions require medical assessments of fitness to drive and require reporting of those who may not be fit to drive such as those who have hypoglycaemia unawareness or severe hypoglycaemia. Diabetes health professionals should be familiar with local guidelines and laws. National organizations have developed detailed guidelines [529].

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16

Disorders Associated with Hypoglycaemia in Children

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KEY LEARNING POINTS

- Normoglycaemia is achieved by complex hormonal and metabolic adaptation processes that control glucose production and utilization.
- Glucose is the major trigger for insulin secretion and, along with the other nutrients and gastrointestinal hormones, provides the signal for insulin release in the prandial phase.
- A single plasma glucose value cannot define hypoglycaemia.
- Hypoglycaemia is a biochemical diagnosis and can occur in a wide variety of conditions.
- Thorough evaluation of the clinical scenario, with measurement of hormonal responses and intermediate metabolites, is vital in identifying the underlying cause and guiding clinical intervention and management
- Hyperinsulinaemic hypoglycaemia (HH) is a heterogeneous condition in terms of the age of onset, clinical presentation, duration, severity, molecular biology, histology and response to medical treatment.
- The risk of brain damage is higher in HH due to lack of alternative fuels. It is recommended that blood glucose concentration be maintained at a widely accepted threshold of >3.5 mmol/L.
- The combination of genetic analysis and 18F-DOPA PET/CT scan has revolutionized the management of Congenital hyperinsulinism (CHI).
- Newer treatments, such as mTOR inhibitors and long-acting octreotide/lanreotide, have been shown to be beneficial for management of diffuse medically unresponsive CHI in small studies and hold promise as possible therapeutic options in the future.
- A diverse spectrum of inherited metabolic defects can present with hypoglycaemia.
- A thorough history and examination should direct differential diagnosis.
- Samples taken at the time of hypoglycaemia are important but most metabolic causes of hypoglycaemia can be made on non-acute samples at time of normoglycaemia.
- Emergency treatment of hypoglycaemia due to an inherited metabolic defect is the same as for any cause of hypoglycaemia.
- Many patients with an inherited metabolic defect causing hypoglycaemia are given an Emergency regimen (ER) consisting of frequent glucose polymer drinks to use during periods of intercurrent illness to prevent or minimize hypoglycaemia.
- Expert advice from a specialist metabolic centre should be sought when managing patients with hypoglycaemia due to an inherited metabolic defect.

Introduction

Hypoglycaemia is common and recently there have been considerable advances in understanding its causes and improvement in its management; nevertheless controversy remains with respect to its definition, glycaemic values that cause complications, prevention and intervention.

Hypoglycaemia results in reduced glucose supply, the principal fuel for most mammalian cells and their metabolic needs. The brain uses glucose as an obligatory substrate under normal conditions but it has a limited supply of endogenous glucose and glycogen and relies on a sustained and continuous supply of glucose from the blood [1]. The maintenance of normoglycaemia is therefore critical for the normal functioning of the brain, especially during active periods of development and growth in infancy and childhood. Conditions that cause hypoglycaemia can cause permanent damage to the brain with long-term neurodevelopmental deficits, which have major implications for children and their carers.

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Physiology of Blood Glucose Control

Blood glucose concentration is tightly regulated by maintaining a balance between glucose production and glucose utilization, and this is achieved by a complex interplay of glucose, insulin and counter-regulatory hormones that include glucagon, catecholamines, growth hormone (GH) and cortisol. Insulin decreases glucose production and increases glucose utilization, whereas glucagon, catecholamines, cortisol and GH increase glucose production and decrease glucose utilization [2].

Glucose Production

Glucose is derived mainly from dietary carbohydrates in the fed state; in the fasted state, blood glucose concentrations are maintained by mobilizing glycogen stores from the liver (glycogenolysis) or production of glucose from non-carbohydrate sources known as gluconeogenesis (liver and kidney) [3].

Glycogenolysis involves breakdown of glycogen to produce glucose and occurs in response to glucagon and/ or adrenaline secretion. This process maintains the blood glucose concentration during fasting and provides the brain with glucose. The first step involves activation of glycogen phosphorylase that phosphorylates glucose molecules in the glycogen chain to form glucose-1-phosphate, which is converted to glucose-6-phosphate. This is then converted to glucose by glucose-6-phosphatase.

Liver, kidneys and muscle store glycogen. Due to the high glycogen content and presence of glucose-6-phosphatase, the liver is the main organ that contributes to glycogenolysis, in comparison with muscle and kidney, which have only small quantities of stored glycogen. In addition, muscle lacks glucose-6-phosphatase required to release glucose into the circulation.

When sufficient energy (ATP) is available, excess glucose is converted to glycogen by glycogen synthase and stored (glycogenesis). The balance between glycogenesis and glycogenolysis is regulated by insulin (favours glycogenesis) and glucagon (favours glycogenolysis). Insulin activates glycogen synthase [4] and inactivates glycogen phosphorylase that causes glycogenolysis [5].

Gluconeogenesis is the production of glucose from non-carbohydrate sources. The main substrates are pyruvate, lactate, glycerol and glucogenic amino acids alanine and glutamine.

In healthy adults, gluconeogenesis contributes 50% of glucose production after an overnight fast and nearly all glucose production after 42 hours [6], but children have limited glycogen stores that sustain blood glucose concentrations for only 12–16 hours. Beyond that period, gluconeogenesis becomes important [7, 8].

Renal Contribution to Glucose Homeostasis

Traditionally the liver was assumed to be the key site of gluconeogenesis, with an insignificant contribution from the kidneys. It is now known that, following an overnight fast, the kidney contributes 20–25% of the glucose released into the circulation [9, 10].

In the postprandial state, hepatorenal glucose reciprocity exists whereby a reduction in hepatic glucose production is compensated by a twofold increase in renal gluconeogenesis [11]. This reciprocity enables replenishment of hepatic glycogen stores and maintains glucose homeostasis in pathological and physiological conditions such as prolonged fasting, liver transplantation and acidosis [12]. Kidneys also contribute to glucose homeostasis by reabsorbing glucose from the proximal convoluted tubules through the sodium glucose transporters (SGLTs) [13].

Glucose Utilization

All tissues use glucose. Insulin contributes by promoting peripheral uptake of glucose and glycogenesis and inhibiting lipolysis and ketogenesis. Increased blood glucose concentrations in the postprandial state are sensed by the β-cell, resulting in increased insulin secretion. Binding of insulin to its receptor in the peripheral tissues causes upregulation of glucose transporters (GLUT) (synthesis and mobilization to cell surface) [14] and enables tissue uptake of glucose.

Following a meal, the rate of gastric emptying influences circulating glucose concentrations. Mixed meals (carbohydrate combined with protein and fat) decrease gastric emptying and stimulate the production of incretins (hormones produced by the gut) and therefore lower postprandial glucose concentrations [15, 16].

Glucose is transported into the cells by carrier-mediated facilitated diffusion. The carriers are a group of proteins known as GLUT. Fourteen have been discovered in humans [17] but the best described are GLUT 1–5 that are crucial for glucose utilization; each of them has a specific tissue distribution and function. GLUT1 and GLUT3 are distributed in all tissues including the nervous system and glucose transport is insulin independent [14]. They have high affinity for glucose and transport glucose readily, which is crucial for ensuring a constant glucose supply to the brain.

GLUT2, a low affinity GLUT, is mainly expressed in the pancreatic β-cells, liver, kidney and small intestine. It plays an important role in maintaining blood glucose concentrations because of its role in glucose absorption from the intestinal lumen, reabsorption of glucose in kidneys, glucose sensing in the pancreatic β-cells and glucose uptake and release in the hepatocytes. The kinetic properties of GLUT2 allow transport of glucose

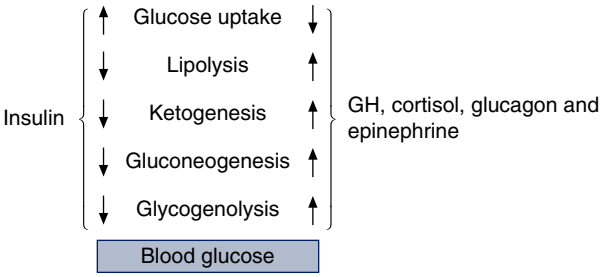

Figure 16.1 Summary of the effects of insulin and counter-regulatory hormones. Insulin reduces blood glucose concentration by inhibiting glycogenolysis, gluconeogenesis, lipolysis and ketogenesis and by enhancing the uptake of glucose by insulin-sensitive tissues. Cortisol, Growth Hormone (GH), glucagon and epinephrine have the opposing effects causing an increase in blood glucose concentrations.

within these tissues even at high blood glucose concentrations. Hence, glucose utilization in these tissues is not dependent on the number and activity of the GLUT but on the blood glucose concentration [18].

GLUT4 is an insulin-dependent GLUT present in the muscle, heart and adipose tissue, and GLUT5 is a fructose transporter in the jejunal brush border [14].

Another family of sodium-dependent glucose transporters (SGLT) located mainly in the luminal surface of the small intestine (SGLT1) and the proximal renal tubule of the kidney (SGLT2) enables energy-dependent glucose uptake [13]. Following uptake, glucose can undergo glycolysis to generate ATP and/or be stored as glycogen (glycogenesis) or fat or converted to lactate. These fates are dependent upon the blood glucose concentration, length of fasting and the hormonal milieu.

In summary, insulin action is geared towards reducing blood glucose concentrations by inhibiting glycogenolysis and gluconeogenesis and by activating glycogenesis and glycolysis. On the other hand, various counter-regulatory hormones including glucagon, adrenaline, cortisol and GH have the opposite action. It is the balance between insulin action and actions of the counter-regulatory hormones that maintains the blood glucose concentration (Figure 16.1).

Role of Gut Hormones in Glucose Homeostasis

A multimodal mechanism of glucose regulation has emerged following the discovery of several hormones released by the gut and elucidation of their impact on blood glucose concentrations.

Incretin hormones glucagon-like peptide 1 (GLP-1) and gastric inhibitory polypeptide (GIP) are the principal incretin hormones produced by the enteric mucosa cells (L and K cells) following a meal. They stimulate insulin secretion and are responsible for the plasma insulin response to an oral glucose load in comparison with an intravenous glucose load (incretin effect) [19]. GLP-1 also delays gastric emptying, inhibits glucagon secretion, promotes satiety and aids weight loss [20].

Ghrelin is secreted by the cells in the fundus of the stomach and acts via the growth hormone secretagogue receptor (GHSR) [21]. Ghrelin concentrations increase before a meal and reduce after [22, 23]. It is a potent stimulator of GH secretion [24] and increases ACTH, cortisol and epinephrine [24, 25], as well as appetite via actions on the hypothalamus [26]. There is a direct effect of inducing glycogenolysis following intravenous infusion of ghrelin [27].

Amylin is a neuroendocrine hormone co-secreted with insulin by the pancreatic β-cell. It enhances insulin actions by suppressing postprandial glucagon secretion [28]. It also slows gastric emptying [29] and reduces food intake and body weight in animal models [30].

Metabolic Adaptation to Birth

In utero, the fetus is exposed to continuous glucose via a carrier-mediated facilitated diffusion across the placenta [31]. Fetal glucose concentrations are a direct reflection of maternal glucose concentrations [32]. Under physiological conditions the fetus produces only minimal amounts of glucose and fetal glucose homeostasis is characterized by high plasma insulin concentrations that stimulate anabolism and glycogen deposition and low glucagon concentrations. However, during prolonged periods of reduced glucose supply, animal studies have shown the ability of the fetus to maintain glucose homeostasis by initiating glycogenolysis and gluconeogenesis at the expense of growth [33]. The fetal brain adapts to chronic hypoglycaemia by modulating GLUT, increasing GLUT1 and decreasing GLUT3, to promote glucose uptake by the brain [34].

After birth, when the continuous supply of glucose is disrupted, the neonate undergoes a period of metabolic and endocrine adaptation for independent extrauterine existence. The blood glucose falls immediately to a nadir in the first 2–4 hours and gradually returns to normal (3.5–5.5 mmol/L) over 48–72 hours. The 'physiological' drop stimulates a counter-regulatory response with increases in glucagon, catecholamines, GH and cortisol within minutes to hours of birth. The rise in glucagon is coupled with the upregulation of glucagon receptors, a fall in insulin concentration and downregulation of the insulin receptors. The resultant decreased insulin–glucagon ratio decreases activity of glycogen synthase and stimulates glycogen phosphorylase thus inhibiting glycogen synthesis and promoting glycogenolysis [35],

the initial primary pathway of glucose production, which is exhausted within a few hours. The reduced insulin–glucagon ratio activates the gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK) providing glucose by 4–6 hours of age, although actual maturation of the enzyme activity to adult levels can take up to 2 weeks [36, 37]. Lipolysis and fatty acid oxidation are induced by the elevated concentrations of catecholamines, thyroid-stimulating hormone [38] and cortisol and by decreased insulin concentrations.

These hormonal changes increase ketogenesis providing ketone bodies as an alternate glucose-sparing fuel for cerebral utilization. Hepatic ketogenesis is limited at birth and remains low during the transitional phase of neonatal hypoglycaemia. Several studies have shown remarkably low ketone bodies associated with hypoglycaemia in the first 24 hours of birth. Term bottle-fed babies have higher blood glucose concentrations than term breastfed babies, who in turn have higher levels of ketone bodies, but this adaptation can take 24–48 hours. This transitional period of hypoketotic hypoglycaemia in neonates may be related to transient dysregulation of insulin secretion with a lower glucose threshold for insulin suppression [39].

GLUT also play a key role in postnatal adaptation. During the immediate postnatal period, there is a predominance of GLUT1 in all tissues. Since GLUT1 is a high affinity GLUT, it increases tissue availability of glucose [40]. GLUT1 gradually decreases after birth and is replaced by specific isoforms in each tissue [41].

The transition to a fed–fasted cycle is accomplished with little consequence in the normal term infant but is often compromised in the premature or SGA infant.

Since the metabolic adaptations can take up to 72 hours, it is difficult to differentiate between physiological and persistent causes of hypoglycaemia during this time. The focus of management during the first 72 hours should be on stabilization of blood glucose concentrations, especially in view of low ketone bodies, and investigations to identify underlying causes should be deferred for 3 days if hypoglycaemia persists.

Metabolic Adaptation to Feeding and Fasting

After a meal, the blood glucose concentration rises. Hyperglycaemia and stimuli from neurogenic and enteroinsular axes lead to increased insulin secretion, which increases glucose utilization and decreases glucose production by inhibiting glycogenolysis and gluconeogenesis (Figure 16.2). In the postprandial phase, insulin also inhibits ketogenesis and lipolysis. Plasma glucose concentration increases within 15 minutes, peaks at around 30–60 minutes and declines until absorption is complete, usually about 4–5 hours later, with plasma insulin concentrations following a similar time course.

The 4–6 hours interval that follows the ingestion of a meal is referred to as the post-absorptive state. A steady state is reached during it whereby glucose production equals glucose consumption and plasma glucose concentrations are maintained within a normal range. Glucose turnover (glucose production and utilization) is then ~10 µmol/kg/min. Non-insulin-dependent utilization of glucose accounts for 80%, mainly by the brain, which accounts for 50% of the total, red blood cells, kidneys and the gastrointestinal system. The glucose concentrations are maintained by interactions between insulin and glucagon, cortisol, GH and catecholamines. Glucagon allows the controlled release of stored glycogen from the liver; insulin restrains the effects of glucagon by preventing accelerated lipolysis and proteolysis; cortisol and GH play permissive roles in setting the sensitivity of the peripheral tissues to glucagon and insulin.

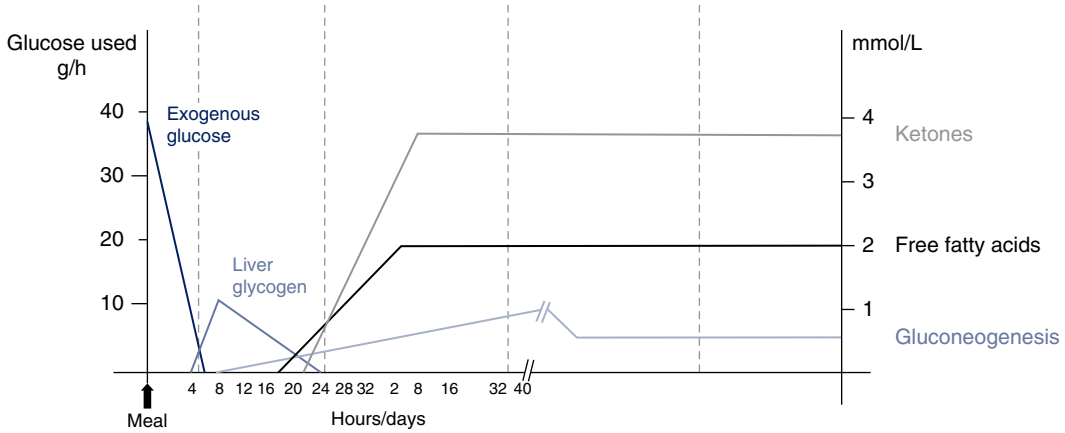

Figure 16.2 Metabolic adaptation to feeding and fasting.

As the period of fast lengthens, insulin secretion decreases and secretion of glucagon and other counter-regulatory hormones increases, which reduces tissue glucose utilization and increases gluconeogenesis, lipolysis and ketogenesis. Glycogenolysis slows down as glycogen stores are exhausted and gluconeogenesis becomes the main process by which glucose is produced. Glucagon secretion with reduced insulin allows stored fats to be converted to glycerol and fatty acids and proteins to be converted to amino acids for gluconeogenesis. The liberated free fatty acids are transported to the liver bound to albumin, where they can either undergo β-oxidation in the mitochondria to yield ketone bodies or be re-esterified to triacylglycerols and phospholipids.

Muscle and other tissues become progressively more dependent on free fatty acids and ketone bodies for their continued energy requirements as the period of the fast is prolonged. Ketone bodies produced in the liver from the oxidation of fatty acids are exported to peripheral tissues as an energy source. They are particularly important for the brain, which has no other substantial non-glucose-derived energy source. Ketone bodies replace glucose as the predominant fuel for nervous tissue, thereby reducing the obligatory requirement of the brain.

The Regulation of Insulin Secretion and Role of K_{ATP} Channels

As insulin is the principal hormone that controls blood glucose concentration, the regulation of its secretion plays a key role in maintaining normoglycaemia. Insulin release is regulated by a complex interplay of nutritional, hormonal and neural factors. Glucose is the major trigger and, along with the other nutrients (amino acids and free fatty acids) and gastrointestinal hormones, provides the signal for insulin release in the prandial phase.

Glucose-Stimulated Insulin Secretion (GSIS)

Glucose induces a biphasic pattern of insulin release. First-phase insulin release occurs within the first few minutes after exposure to an elevated glucose concentration; this is followed by a more sustained second phase of insulin release.

In the pancreatic β-cells, glucose enters the cytoplasm through facilitative GLUT, especially the glucose transporter 2 (GLUT2) [42, 43]. GLUT2 has a high Km for glucose (~40 mmol/L) and is expressed predominantly in the liver, kidney, β-cells of the pancreas and intestinal mucosal cells. The high Km for glucose of GLUT2 allows glucose transport by pancreatic β-cells and hepatocytes in proportion to the blood glucose concentration [42, 44]. Glucose entry into the cells is followed by phosphorylation by a highly glucose-specific enzyme, glucokinase. Glucokinase acts as a glucose sensor and, due to its high Km, it does not saturate under physiological basal glucose levels. This allows the β-cells to adjust the rate of insulin secretion and is thought to be the rate-limiting step for insulin secretion [45]. Following phosphorylation, glucose is metabolized to pyruvate (glycolysis), which then enters the Krebs cycle to produce ATP. ATP triggers closure of the adenosine triphosphate-sensitive potassium channels (K_{ATP}) located on the β-cell membrane, which leads to insulin release.

The K_{ATP} channels are hetero-octameric complexes consisting of four inwardly rectifying potassium channels (Kir6.2) and four sulphonylurea receptor 1 (SUR1) subunits. The Kir6.2 forms the pore of the channel and the SUR1 (an ATP-binding cassette transporter) acts as a regulatory subunit [46]. K_{ATP} channels are regulated by adenine nucleotides to convert changes in cellular metabolic levels to membrane excitability.

K_{ATP} channels can function only if they are assembled and transported correctly to the cell membrane surface (trafficking). The assembly and trafficking of K_{ATP} channels are intricately linked. Only octameric K_{ATP} channel complexes are capable of expressing on the cell membrane surface. For example, Kir6.2 and SUR1 both possess an endoplasmic reticulum (ER) retention signal (RKR) that prevents the trafficking of each subunit to the plasma membrane in the absence of the other subunit [47]. Co-expression of two subunits masks these retention signals, allowing them to move to the plasma membrane. The retention signal is present in the C-terminal region of Kir6.2 and in an intracellular loop between transmembrane domain 1 (TMD1) and nucleotide-binding domain 1 (NBD1) in SUR1. Truncation of the C-terminus of Kir6.2 deletes its retention signal, allowing functional expression of Kir6.2 in the absence of the SUR1 subunit [48]. In addition to these retrograde signals, the C-terminus of SUR1 has an anterograde signal, composed in part of a dileucine motif and downstream phenylalanine, which is required for K_{ATP} channels to exit the ER/cis-Golgi compartments and transit to the cell surface [49]. Deletion of as few as seven amino acids, including the phenylalanine, from SUR1 markedly reduces surface expression of K_{ATP} channels [50]. Thus, one function of SUR1 is as a chaperone protein to facilitate the surface expression of Kir6.2.

There is also some evidence that Kir6.2 provides a reciprocal service for SUR1 [51]. The SUR1 protein shows a high affinity binding capacity to the sulphonylurea glibenclamide, indicating that SUR1 confers sulfonylurea binding [52]. The sulfonylurea drugs (glibenclamide and tolbutamide) inhibit the channels and are used in the treatment of non-insulin-dependent (type II) diabetes mellitus. The other class of drugs, known as potassium

channel openers (e.g. diazoxide), activates the channel and are used to suppress insulin secretion. In euglycaemic conditions, the K_{ATP} channels are open, which allows potassium efflux from the pancreatic β-cell. This keeps the β-cell membrane at a negative potential at which voltage-gated calcium (Ca^{2+}) channels are closed. An increase in the blood glucose concentration leads to production of ATP. The increase in the ATP/ADP ratio triggers closure of the K_{ATP} channels leading to the depolarization of the β-cell membrane, which in turn leads to the opening of the voltage-gated Ca^{2+} channels and Ca^{2+} ion influx. The entry of calcium ions triggers exocytosis of insulin (Figure 16.3).

Apart from the above described K_{ATP} channel-dependent pathway, glucose also stimulates insulin secretion via K_{ATP} channel-independent pathways [54]. The K_{ATP} channel-independent pathways act synergistically to augment the secretory responses to increased intracellular calcium and lead to the second and more sustained phase of glucose-stimulated insulin secretion. However the mechanisms of these pathways are poorly defined.

Definition of Hypoglycaemia

Fasting blood glucose is maintained within a range of 3.5–5.5 mmol/L, except in the first 72 hours of life. In the postprandial phase, blood glucose may increase transiently but quickly reverts to this range. In healthy adults, symptoms of hypoglycaemia occur at a mean plasma glucose value of 3 mmol/L, and hypoglycaemia is defined as a plasma glucose concentration low enough to cause neuroglycopenic or autonomic symptoms relieved on normalization of plasma glucose (Whipple's triad). This definition holds true for older children who can reliably recognize and communicate the symptoms but not in younger children and neonates for several reasons [55].

Firstly, the signs and symptoms of hypoglycaemia in neonates and infants can be non-specific – such as poor feeding, lethargy, apnoea and irritability – and variable. When hypoglycaemia is a part of an underlying process (e.g. hypoxic ischaemic encephalopathy, sepsis), the clinical signs are likely to be indistinguishable from

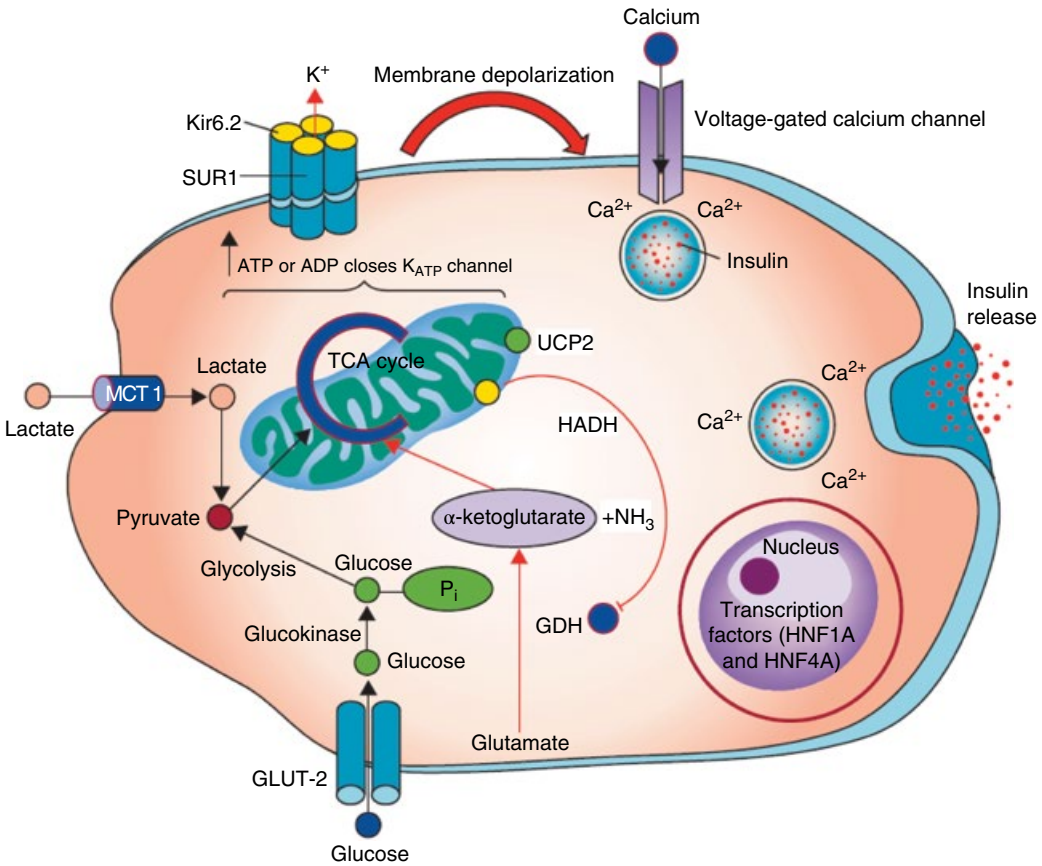

Figure 16.3 Outline of the pancreatic β-cell showing the role of K_{ATP} channels in regulating insulin secretion. β-Cell K_{ATP} channels play a key role in transducing the metabolic signals generated from glucose metabolism to changes in plasma membrane electrical activity and insulin secretion. *Source:* Figure adapted from Shah P et al. [53]. (*See insert for colour representation of the figure.*)

those of the underlying condition. If one were to define hypoglycaemia as a plasma glucose that leads to neurological impairment, it must be borne in mind that brain injury is related not only to the plasma glucose value but also to the presence or absence of alternative fuels and existing co-morbidity. The risk of brain injury is higher in conditions such as hyperinsulinism and fatty acid oxidation defects, where production of alternative fuels for cerebral metabolism is suppressed. Co-morbid conditions such as sepsis, hypoxia and seizures that increase cerebral glucose demand have an additive effect on the resultant brain injury [56] and so the same plasma glucose value may or may not meet metabolic demands in different situations. The glycaemic threshold for neurological responses varies across a range of plasma glucose values and also depends on the duration and frequency of hypoglycaemia. Thus no single plasma glucose value can define hypoglycaemia.

Finally, transient hypoglycaemia in the first 48 hours after birth is common and may represent a physiological adaptive process. Several studies have shown that low plasma glucose values (widely accepted as <2.6 mmol/L) are not uncommon in term healthy infants, especially in the first 2–3 hours after birth [57–65]. This is followed by a spontaneous increase in blood glucose concentration, even in the absence of nutrition.

Therefore, using any numerical cut-off to define neonatal hypoglycaemia is not scientifically justifiable and the definition must be tailored to the individual infant and based on the clinical situation.

It is also important to consider the possibility of artefactual hypoglycaemia when interpreting a glucose value. Point-of-care meters are convenient screening tools but the diagnosis must be confirmed by a clinical laboratory method. Also, arterial blood glucose values are higher than venous ones and plasma/serum glucose values are higher than whole blood glucose values. Finally, delay in analysing samples gives lower values due to red cell glycolysis. Prompt measurement should be undertaken with the sample in a fluoride oxalate collection tube to block post-sampling glucose metabolism.

Screening

Routine screening and monitoring of blood glucose concentration is not recommended for term healthy infants born after a normal pregnancy and delivery. Monitoring blood glucose concentrations is recommended in an infant who is clinically unwell, or if there are clinical signs compatible with hypoglycaemia, or in the presence of risk factors for hypoglycaemia. Data on the timing and intervals for glucose screening are limited but it is accepted that, as the aim of blood glucose monitoring is to identify the lowest measurement, pre-feed measurements should be undertaken. The clinically unwell infant and those with signs of hypoglycaemia must have blood glucose measured immediately. Screening of the asymptomatic at-risk infant should be performed within the first few hours of birth and continued through multiple feed–fast cycles.

Numerical Cut-Offs Used in Clinical Practice

In a survey of 178 pediatricians with examination of 36 textbooks, the definition of hypoglycaemia varied from <1 to <4 mmol/L [66] and the quest for an ideal glucose threshold continues. Since the aim of identifying and treating hypoglycaemia is to maintain cerebral metabolism, defining hypoglycaemia in relation to brain function is most appropriate. A study of 17 children (only 5 neonates) identified abnormalities of brainstem auditory or somatosensory evoked potentials among some children when their blood glucose concentrations fell below 2.6 mmol/L (47 mg/dL) [67]. These effects, observed not only on auditory evoked response but also on electroencephalogram signals and visual evoked potentials, were not reproduced in other studies [68]. Another study, marked by its large sample size and statistical power, reported a strong association between the number of days (exceeding 5) on which blood glucose concentrations of <2.6 mmol/L were documented and lower Bayley developmental scores at 18 months of age [69]. However, only preterm infants were studied and their immature counter-regulatory responses might make them more vulnerable to the effects of hypoglycaemia. These results cannot be extrapolated to other groups of infants. Furthermore, the outcomes were not sustained at later assessments at 7.5–8 years of age [69]. The results of these studies have often been misinterpreted as identification of a numerical threshold (<2.6 mmol/L) at which brain injury is likely to occur. In support of the glucose threshold of 2.6 mmol/L is a recent large prospective study of at-risk term and late-preterm infants (404 participants) [70]. In this study, neonatal hypoglycaemia was not associated with adverse neurodevelopmental outcomes at 2 years with a treatment threshold of 2.6 mmol/L. The paper concluded that, in the main at-risk group of infants, frequent screening and intervention aimed at maintaining a blood glucose concentration of at least 2.6 mmol/L is effective in preventing neuronal injury in at-risk term and late-preterm newborns. The rates of neurodevelopmental delay were high in those who experienced hypoglycaemia (33%) and those who did not (36%), making the argument for higher glucose concentration thresholds. Having control data on healthy term children would have helped substantiate the claims.

In the absence of a clear definition of hypoglycaemia, operational thresholds for intervention have been recommended that merely provide guidance [71]. Intervention is recommended in symptomatic infants if blood glucose is <2.5 mmol/L; intervention is suggested in asymptomatic infants at a blood glucose concentration <2.0 mmol/L; intravenous glucose infusions should be used at glucose concentrations between 1.1 and 1.4 mmol/L. A recent Pediatric Endocrine Society (PES) consensus workshop [55] agreed that the evidence was still lacking to specify a range of glucose concentration that would define significant hypoglycaemia and recommended higher glucose target thresholds to prevent brain damage and ensuing disability. In the first 48 hours after birth, the target threshold was 2.8 mmol/L (50 mg/dL); after 48 hours of age, the glucose target was raised to >3.3 mmol/L (>60 mg/dL); for neonates with a suspected congenital hypoglycaemic disorder (such as hyperinsulinism) and older infants and children with a confirmed hypoglycaemic disorder, the goal of treatment to maintain a plasma glucose concentration >3.9 mmol/L (70 mg/dL) was recommended [55].

None of the numerical thresholds defined are robust and provide only guidance. The decision to intervene and maintain specific glucose concentrations should be based on the individual clinical situation.

Aetiology and Clinical Approach to Hypoglycaemia

Despite the urgent requirement to treat hypoglycaemia, an initial assessment of history, examination and the collection of critical blood and urine samples are vital. Treatment should proceed with oral or intravenous glucose. Hypoglycaemia is often overlooked because the history and signs may be vague and non-specific, such as poor feeding, lethargy and irritability, particularly in the neonate and infant. A high level of suspicion is required in a neonate presenting with apnoeic events, twitches or other subtle signs but many hypoglycaemic infants are asymptomatic, which has led to routine screening in at-risk infants, such as infants of a diabetic mother, babies born with extreme intrauterine growth retardation and babies with hypothermia.

Studies in adults have clearly defined the progressive development of symptoms and signs of hypoglycaemia using hyperinsulinaemia-induced hypoglycaemia. As the blood glucose concentrations are artificially lowered, there is a classic progression from hunger and food seeking to onset of sympathetic nervous system activation with the associated features of sweating, tremor and tachycardia. As glucose concentrations fall further, impairment of cognitive function occurs with confusion, mental slowness, inappropriate behaviour, loss of consciousness, generalized convulsions and death.

There are many causes of hypoglycaemia (Table 16.1). A thorough history and examination are vital in delineating the underlying cause and planning further investigations and management. The history should focus on episodes of hypoglycaemia, the timing of such events in relation to food and the presence of predisposing and associated factors. The time from the last meal to the onset of hypoglycaemia is important in determining the underlying cause. Poor fasting tolerance for age could suggest increased utilization of glucose because of high insulin concentrations e.g. congenital hyperinsulinism (CHI) or a form of GSD. However, onset of hypoglycaemia after 2–3 hours post-meal could possibly suggest post-prandial hyperinsulinaemic hypoglycaemia (either protein-sensitive or Dumping syndrome), hereditary fructose intolerance or galactosaemia. Conditions associated with onset of symptoms >4 hours after the last meal are those that involve defects in glycogenolysis, gluconeogenesis, production of free fatty acids and ketone bodies or lack of counter-regulatory hormones. Rare conditions, such as mitochondrial disorders linked with intracellular energy deprivation, may present with symptoms of hypoglycaemia with a normal plasma glucose concentration (pseudohypoglycaemia).

Age of Onset

Severe neonatal hypoglycaemia with high glucose requirement >8 mg/kg/min generally suggests a congenital defect in insulin release, whereas hypoglycaemia on days 1 and 2 of life managed with increased feeds would be typical of a baby born with deficiency of counter-regulatory hormones such as cortisol or GH. Babies with hypopituitarism can also present with prolonged jaundice. Presentation of the first episode of hypoglycaemia in infancy after weaning and prolongation of fasting to 8 hours raises the possibility of defects in the gluconeogenic or fat metabolism pathways. Children presenting for the first time with hypoglycaemia in mid-childhood raise questions of common disorders, such as ingestion of alcohol, or extremely rare conditions, such as insulinoma or exercise-induced hypoglycaemia.

Past History

A critical review may reveal missed events that suggest the duration of hypoglycaemia may be longer than first thought. The child may often be sweaty, shaky, cold and clammy early in the morning and mood and general cognition improves after breakfast. The diagnosis of idiopathic epilepsy may need to be revised with the discovery of hypoglycaemia.

Table 16.1 Causes of hypoglycaemia.

Hyperinsulinism	• ***Transient*** Infant of diabetic mother Perinatal asphyxia Rhesus haemolytic disease Intrauterine growth restriction (IUGR)/large for gestational age • ***Congenital (see Table 16.5)*** *ABCC8/ KCNJ11/ GCK/ GLUD1/ HADH/ HNF4A/HNF1A/ UCP2/ SLC16A1* • ***Insulinoma*** • ***Other syndromes associated with Hyperinsulinism (see* Table *16.4)***
Hypoinsulinaemic hypoketotic hypoglycaemia	• Activating *AKT2* mutations • Abnormal processing of IGF-II – non-islet cell tumour hypoglycaemia (NICTH) • Factitious hypoglycaemia
Counter-regulatory hormone deficiency	• Growth hormone deficiency • Adrenal insufficiency • Panhypopituitarism
Disorders of glycogen synthesis and breakdown (glycogen storage disease [GSD])	• GSD Ia (glucose-6-phosphatase deficiency), GSD Ib (glucose-6-phosphatase transporter protein defect or GSD type I non-a) • GSD III (amylo-1,6-glucosidase deficiency) • GSD IX (phosphorylase kinase deficiency) • GSD 0 (glycogen synthase deficiency)
Disorders of fatty acid oxidation and ketone synthesis	• Medium chain acyl-CoA dehydrogenase deficiency (MCADD) • Long chain hydroxyacyl-CoA dehydrogenase deficiency (LCHADD) • Very long chain acyl-CoA dehydrogenase deficiency (VLCADD) • Mitochondrial trifunctional protein deficiency • Multiple acyl-CoA dehydrogenase deficiency • Carnitine transporter and carnitine cycle disorders • HMG (3-hydroxy-3-methylglutaryl) CoA synthase deficiency • HMG (3-hydroxy-3-methylglutaryl) CoA lyase deficiency
Disorders of gluconeogenesis	• Fructose-1,6-bisphosphatase deficiency • Phosphoenolpyruvate carboxykinase (PEPCK) deficiency • Pyruvate carboxylase deficiency
Glucose transporter defects	• GLUT (glucose transporters) 1/2/3 defects • Fanconi Bickel syndrome (GLUT2 deficiency)
Mitochondrial respiratory chain disease	
Branched-chain organic aciduria	• Methylmalonic aciduria • Propionic aciduria
Other metabolic conditions	Galactosaemia, fructosaemia, tyrosinaemia, glutaric aciduria type 2, maple syrup urine disease, propionic acidaemia
Other causes	• Abrupt cessation of glucose infusion • Exchange transfusion without glucose replacement • Drugs (e.g. THAM,[a] maternal β-blockers) • Idiopathic ketotic hypoglycaemia (diagnosis of exclusion – noted in children when unwell) • Post-diabetic hypoglycaemia (hypoglycaemia in diabetes mellitus)

[a] THAM, tromethamine; tris-hydroxymethylaminomethane.

Pregnancy, Birth and Neonatal History

Premature infants have an increased frequency of hypoglycaemia compared with term infants, as do babies born small for gestational age. Pregnancy affected by diabetes of any type, particularly if glucose control was suboptimal, leads to increased risk of neonatal hypoglycaemia and of macrosomia, perhaps leading to a difficult or prolonged delivery. Babies born by a traumatic and/or difficult delivery

associated with hypoxia are at risk of hypoglycaemia. Polycythaemia in the neonatal period is associated with hypoglycaemia and often resolves with exchange transfusion. In the neonatal period, symptoms and signs of hypoglycaemia are vague and non-specific, such as jitteriness, apnoea, cyanosis, floppiness and jaundice.

Family History

In some populations, there is an increase in the prevalence of inherited causes of hypoglycaemia. Predisposing factors, such as adrenal dysfunction, can also be inherited and need to be noted.

Dietary and Drug History

The relationship between hypoglycaemia and timing of the last meal is important. Hypoglycaemia may be triggered by certain types of food, such as high protein load, high fructose content, toxin of tropical fruit after consumption of unripe ackee fruit (as seen in Jamaican vomiting sickness) and high glycaemic index foods, which may lead to rebound hypoglycaemia. A number of drugs and chemicals (e.g. alcohol, aspirin, oral hypoglycaemic agents, insulin injections, β-blockers and quinine) may interrupt intermediate metabolism leading to episodes of hypoglycaemia.

Examination

Initial examination should record height, weight, body mass index and, in the neonate, features suggesting macrosomia, small for gestational age and prematurity. Dysmorphic features may suggest inborn errors of metabolism, midline defects such as cleft lip/palate and optic nerve hypoplasia associated with hypopituitarism and organomegaly associated with Beckwith–Wiedemann syndrome. Other relevant findings are hyperventilation, suggesting metabolic acidosis linked with metabolic disorders, hyperpigmentation associated with adrenocorticotropic hormone (ACTH) excess and, in the neonate, ambiguous genitalia linked with cortisol deficiency. A micropenis with bilateral undescended testes and/or conjugated hyperbilirubinaemia may suggest a diagnosis of congenital hypopituitarism. Liver enlargement is a clue to some disorders of glycogen storage.

The child presenting late with hypoglycaemia may demonstrate evidence of the deleterious effects of previous hypoglycaemic episodes, such as delayed development, behavioural disorders, hemiplegia or blindness.

Investigations for Hypoglycaemia

Hypoglycaemia in the first 2–3 days of life is common and the focus of management during this transitional phase should be on maintenance of blood glucose concentrations rather than investigating an underlying cause.

Urgent Investigations

A hypoglycaemia screen (Table 16.2) or 'critical sample' at the time of hypoglycaemia is vital in the evaluation of hypoglycaemia. Where possible, it should be carried out at presentation and before treatment to identify the aetiology of hypoglycaemia. A critical sample should include plasma glucose, insulin/C-peptide, ketones (β-hydroxybutyrate [BOHB]), fatty acids (non-esterified fatty acids [NEFA]) and lactate as a minimum. Low concentrations of cortisol and/or GH during hypoglycaemia do not exclude or confirm deficiency and need validation with growth measurements and further testing.

If blood samples at the time of hypoglycaemia are difficult to obtain, the child should be treated with rapid correction of the hypoglycaemia, and a fast provocation test can later be planned to understand the cause of hypoglycaemia.

Fast Provocation Test

Children are able to fast if they have intact glycogenolytic, lipolytic, gluconeogenic and ketogenic pathways. Their hormonal regulation adaptation to fasting helps maintain blood glucose and produce alternate energy substrates. Hence one can investigate for conditions affecting the hormonal regulation and metabolic pathways during a period of controlled fasting.

Table 16.2 Investigations in hypoglycaemia of unknown cause, the 'hypoglycaemia screen' [92].

Glucose	Blood spot or plasma acylcarnitine profile[a]
Insulin	Plasma amino acids[a]
Cortisol	Ammonia[a]
C-peptide	Urine ketones and organic acids (preferably first void urine after hypoglycaemic episode)[a]
Growth hormone	
Free fatty acids	
Ketones (β-hydroxybutyrate)	
Lactate	
Blood gas	

[a] Sample may be taken after treatment of hypoglycaemia.

Good intravenous access must be obtained before the test [93]. Disorders of fatty acid oxidation should have been excluded on acylcarnitine profile before commencing a fast provocation test since toxic metabolites may accumulate before onset of hypoglycaemia. Careful supervision of the test is essential, especially in infants with suspected CHI because they can suddenly and rapidly become hypoglycaemic.

All feeds and or intravenous fluids are withheld and only intake of water is permitted during the test. Blood glucose is monitored frequently and blood samples (hypoglycaemia screen; Table 16.2) collected when hypoglycaemia (many centers use a cut-off of capillary blood glucose <3.0 mmol/L or 53 mg/dL) or symptoms appear, followed by correction of hypoglycaemia.

The duration of fasting depends on the age of the child. Generally, upto 6 hours is deemed appropriate in a full-term neonate, 8 hours at 6 months of age, 12 hours at 1 year, 18 hours at 1.5–2 years, 20 hours at 2–8 years and 24 hours over 8 years.

Further investigations are detailed in Table 16.3.

Other Planned Investigations

In some children, further provocative tests may have to be undertaken to understand or confirm the underlying trigger for hypoglycaemia. These tests should be conducted under supervision and in tertiary centres due to the risk of severe hypoglycaemia.

In children with suspected protein sensitivity, a protein/leucine load may confirm suspicion and help plan management. This is an important feature of CHI due to mutations in *GLUD1* and *HADH* but may also be seen in some patients with mutations in *ABCC8* gene [94].

An exercise tolerance test can help unmask exercise-induced hypoglycaemia due to mutations in *SLC16A1* gene [95]. *SLC16A1* encodes monocarboxylate transporter 1, which is required for transport of pyruvate and lactate (see Table 16.5). Dominant activating mutations in *SLC16A1* increase pyruvate uptake during strenuous anaerobic exercise and pyruvate-stimulated insulin secretion [95]. Hypoglycaemic episodes may be prevented by avoiding strenuous exercise.

Postprandial hyperinsulinaemic hypoglycaemia (PPHH) refers to the development of hypoglycaemia within a few hours of meal ingestion. It is associated with inappropriate insulin secretion in response to the meal. The most common cause is the 'dumping' syndrome in infants who have undergone Nissen's fundoplication or gastric bypass surgery [96]. It has been observed that children with PPHH after Nissen's fundoplication have abnormally exaggerated secretion of glucagon-like peptide-1 (GLP-1), which may contribute to the exaggerated insulin surge and resultant hypoglycaemia. PPHH may be investigated by mixed meal test

Table 16.3 More detailed investigations (depending on the suspected cause).

Suspected congential hyperinsulinism	Genetics, 18F-DOPA PET/CT scan and specific provocation tests may be indicated depending on clinical phenotype (please refer to section 'Hyperinsulinaemic hypoglycaemia')
Suspected insulin-like action (hypoketotic hypoinsulinaemic hypoglycaemia)	Activating *AKT2* mutation; exome sequencing for *AKT2;* abnormal IGF-II isoforms
Suspected hormonal cause	GH: growth hormone stimulation test, serum IGF1 and IGFBP3; ACTH/cortisol: short Synacthen test, ACTH; very long chain fatty acids (adrenal insufficiency as part of X-linked adrenoleukodystrophy)
Suspected glycogen storage disease (GSD)	Cholesterol/triglycerides; urate; LFTs; GSD exome sequencing
Suspected fatty acid oxidation disorder	Fatty acid flux studies (skin biopsy for fibroblast culture)
Suspected gluconeogenesis defect	Leucocyte fructose-1,6-bisphosphatase activity; liver phosphorylase
Suspected hepatic glycogen synthesis disease	Pre- and postprandial lactate
Suspected mitochondrial respiratory chain disease	Elevated lactate, normal or high creatine kinase; ECHO to look for cardiomyopathy; renal tubular screen; exome sequencing for mitochondrial disorders
Suspected ketone body synthesis or utilization disorder	HMG-CoA mutational analysis; liver biopsy
Suspected other metabolic causes	Red cell galactose-1-phophate uridyltransferase activity; plasma amino acids; glycerol; transferrin isoelectric focussing (CDG syndromes); pyruvate/acetoacetate
Unexplained	Urine toxicology Proinsulin/preproinsulin Check for insulin receptor mutation (Donohue syndrome)

[97], oral glucose tolerance test or the standardized hyperglucidic breakfast test [98].

Emergency Management of Hypoglycaemia

Prompt management of hypoglycaemia is vital to avoid its complications. Immediate management includes oral administration of glucose gel (one-third tube in neonates to one tube in older children – one tube containing 25 g of carbohydrate). Older children can also be given oral glucose-containing drinks or glucose tablets (1–3 tablets of 4 g each) if conscious. In case of altered consciousness, intramuscular glucagon 1 mg or 2 mL/kg of 10% glucose bolus given intravenously will raise blood glucose concentrations promptly (Table 16.6). It is important that blood glucose concentration is measured after 15 minutes to confirm restoration to normal.

Causes of Hypoglycaemia

Hyperinsulinaemic Hypoglycaemia

Hyperinsulinaemic hypoglycaemia (HH) is a clinically, pathologically and genetically heterogeneous disease characterized by dysregulation of insulin secretion from pancreatic β-cells. HH occurs in neonates, children and adults but the underlying mechanisms differ. It is the most common cause of persistent hypoglycaemia in neonates and is associated with an inappropriate elevation of serum insulin, C-peptide and proinsulin concentrations [99].

Under a normal physiological state, pancreatic β-cells secrete the amount of insulin required to maintain a normal blood glucose concentration. The unregulated secretion of insulin in patients with HH drives glucose into the insulin-sensitive tissues (skeletal muscle and adipose tissue), causing hypoglycaemia that is compounded by the fact that insulin inhibits glucose production by inhibiting glycogenolysis, gluconeogenesis, lipolysis and ketogenesis. Hence the brain is deprived of both its primary and secondary energy sources (glucose and ketone bodies). If left untreated, HH can lead to brain damage with associated complications like epilepsy, cerebral palsy and neurological impairment or death secondary to hypoglycaemia [100]. There are various conditions and aetiological causes of HH (Table 16.1). HH is associated with intrauterine growth retardation, perinatal asphyxia, rhesus isoimmunization and maternal diabetes mellitus. In these conditions, HH is diazoxide responsive and usually transient but some infants with HH associated with IUGR and perinatal asphyxia can have more persistent HH, requiring diazoxide treatment for months. There are also syndromes that can cause HH (Table 16.4). A rare cause of HH is an insulinoma, which may occur sporadically or in association with MEN1. HH may also present in the postprandial period, such as in children after gastro-oesophageal surgery (dumping syndrome) or rarely with insulin receptor gene defects.

HH can be congenital (CHI) due to mutations in genes implicated in the pathway of insulin secretion, of which nine are currently implicated [101]. The phenotypic features of these genetic causes are summarized in Table 16.5. Clinically, it is useful to differentiate CHI into diazoxide responsive and unresponsive forms. Histologically, CHI is classified mainly into diffuse, focal and atypical (limited islet nuclear enlargement [LINE]) forms. Diffuse disease, inherited in an autosomal recessive or dominant form, is the more common form

Table 16.4 Syndromes associated with hyperinsulinaemic hypoglycaemia [86–91].

Pre- and postnatal overgrowth syndromes
- Beckwith–Wiedemann syndrome: 11p15.5, 11p15.4
- Sotos syndrome: *NSD1* (5q35)
- Simpson–Golabi–Behmel syndrome: *GPC3* (Xq26), *GPC4* (Xp22)
- Perlman syndrome: *DIS3L2* (2q37)

Chromosomal abnormality syndromes
- Patau syndrome: trisomy 13
- Mosaic Turner syndrome: (loss of X in some cells)

Postnatal growth failure syndromes
- Kabuki syndrome: *KMT2D* (12q13), *KDM6A* (Xp11.3)
- Costello syndrome: *HRAS* (11p15)

Contiguous gene deletion affecting the *ABCC8* gene
- Usher syndrome: *USH1C* (11p15.1)

Syndromes leading to abnormalities in calcium homeostasis
- Timothy syndrome: *CACNA1C* (12p13.33)

Congenital disorders of glycosylation syndromes (CDG)
- Congenital disorder of glycosylation 1a, 1b, 1d: *PMM2* (16p13.2), *MPI* (15q24.1), *ALG3* (3q27.1)

Insulin resistance syndrome (insulin receptor mutation)
- Donohue syndrome (leprechaunism): *INSR* (19p13)

Others
- Congenital central hypoventilation syndrome: *PHOX2B* (4p13)
- Adenosine kinase deficiency
- Mutations in calcium voltage-gated channel subunit alpha1 D: *CACNA1D* (3p21.1)
- Mutations in forkhead box protein A2: *FOXA2* (20p11.21)
- Mutations in phosphomannomutase 2: *PMM2* (16p13.2)
- Mutations in phosphoglucomutase 1: *PGM1* (1p31.3)

Table 16.5 Genetic aetiology of CHI.

ABCC8 (SUR1)[a]*/ KCNJ11 (Kir6.2)*[a]	*ABCC8* – sulfonylurea receptor (SUR1) gene and *KCNJ11*; inwardly rectifying potassium channel (Kir6.2) gene. The K_{ATP} channel is a heteromultimeric complex of at least two proteins designated SUR1 (*ABCC8* gene) and Kir6.2 (*KCNJ11* gene). The Kir6.2 and SUR1 subunits are encoded by the genes *KCNJ11* and *ABCC8* (both genes localized to chromosome 11p15.1), respectively, mutations that result in CHI [72]. Recessive inactivating mutations in the *ABCC8* and *KCNJ11* genes are the most common causes of medically unresponsive diffuse CHI [72, 73]. Dominant inactivating mutations in *ABCC8* and *KCNJ11* usually cause CHI with a milder phenotype, although medically unresponsive forms have been reported recently [74]
GCK	Glucokinase (*GCK*) gene – glucokinase is a key regulator enzyme in pancreatic β-cells and is referred as a pancreatic β-cell sensor [75]. *GCK* mutations can lead to variable phenotypes ranging from symptomatic hypoglycaemia responding to standard medical therapy to medically unresponsive severe HH
GLUD1	Glutamate dehydrogenase (GDH) is an intra-mitochondrial enzyme encoded by *GLUD1* on chromosome 10q23.3. This is associated with hyperinsulinism/hyperammonaemia (HI/HA) syndrome. HI/HA syndrome usually responds to diazoxide [76]
HADH	Mitochondrial oxidation enzyme short chain L-3-hydroxyacyl-coenzyme A dehydrogenase (SCHAD), encoded by *HADH* (hydroxyacyl-coenzyme A dehydrogenase) catalyses the penultimate step in fatty acid β-oxidation in the mitochondria. Defects in SCHAD can cause protein-sensitive HH [77]. Patients may have raised plasma hydroxyl-butyrylcarnitine and urinary 3-hydroxyglutarate levels [78]. CHI due to *HADH* mutation usually responds to diazoxide
HNF4A	Hepatocyte nuclear factor 4-alpha is a member of the nuclear receptor family of transcription factors. The *HNF4A* gene is highly expressed in the liver, kidney, gut and pancreatic islets. Heterozygous mutations in *HNF4A* have been reported to lead to a dominantly inherited condition with a dual phenotype of HH in the neonatal period and MODY1 in adult life [79]. *HNF4A* mutation may also cause renal Fanconi tubulopathy [80] and usually responds to diazoxide
HNF1A	Hepatocyte nuclear factor 1-alpha is another transcription factor thought to play an important role in the expression of several genes involved in glucose-stimulated insulin secretion [81]
SLC16A1	Solute carrier family 16, member 1. Exercise-induced hyperinsulinism (EIHI) is an autosomal dominant disorder in which strenuous exercise causes HH. Heterozygous gain-of-function mutations in the solute carrier SLC16A1 that encodes monocarboxylate transporter 1 (MCT1; required for transmembrane transport of pyruvate and lactate) cause EIHI [82–84]. Most patients can be managed by avoiding strenuous exercise and treating with diazoxide
UCP2	Mitochondrial uncoupling protein 2 (UCP2), a member of the UCP family, is widely expressed in tissues, including pancreatic islets. This protein plays an important role in regulating TCA cycle intermediates and negative regulation of β-cell insulin secretion [85]. Inactivating mutations of UCP2 cause dysregulated insulin secretion

[a] *ABCC8* and *KCNJ11* play key roles as components of the pancreatic β-cell K_{ATP} channel in regulating insulin secretion. Mutations in these genes account for >50% of congenital hyperinsulinism of infancy (CHI).

of CHI and affects the entire pancreas [102]. In atypical/LINE forms of HH, the histological abnormalities may be diffuse with the presence of normal and abnormal islets [103]. Focal disease is usually sporadic in nature and HH is the result of abnormal insulin secretion from a certain area of the pancreas. Focal lesions can be cured by removal of the affected area in contrast with medically unresponsive diffuse disease, which requires near-total pancreatectomy.

Diagnosis of Hyperinsulinaemic Hypoglycaemia

The diagnosis of HH is based on clinical presentation and detection of the characteristic biochemical profile, namely, hypoketonaemic, hypofattyacidaemic hypoglycaemia, arising from the anabolic effects of excessive insulin action at the time of hypoglycaemia. Clinical clues for diagnosis of HH include macrosomia or severe IUGR and high glucose requirement (>8 mg/kg/min, normal range of 4–6 mg/kg/min) to maintain normoglycaemia.

The characteristic metabolic profile can be identified either during spontaneous hypoglycaemia or hypoglycaemia induced by provocation tests (e.g. controlled fast, exercise or protein ingestion). Provocation tests should be done under controlled conditions with close monitoring of blood glucose as it can be potentially life threatening. Laboratory findings at the time of hypoglycaemia will include detectable insulin with inappropriately low fatty acids and ketone bodies. Table 16.6 shows biochemical markers that help to diagnose HH. Under normal physiological conditions,

Table 16.6 Biochemical markers that help in the diagnosis of HH (when blood glucose <3 mmol/L [53 mg/dL] on glucose infusion rate of >8 mg/kg/min) [92, 99].

Blood samples	Confirmation of diagnosis of HH • Detectable serum insulin and/or C-peptide (in endogenous HH) • Suppressed/low serum ketone bodies • Suppressed/low serum free fatty acids Other investigations during hypoglycaemia screen may show • Low branched-chain (leucine, isoleucine and valine) amino acids • Elevated plasma hydroxybutyrylcarnitine (*HADH* mutation) • Elevated ammonia (*GLUD1* mutation)
Urine samples	• Negative ketone bodies • Elevated C-peptide • Elevated urinary 3-hydroxyglutarate (elevated in *HADH* deficiency)

insulin production should be switched off during hypoglycaemia. As insulin release is pulsatile in nature and has a short half-life, measurement of C-peptide, which has a longer half-life and reflects endogenous insulin production, can be helpful when the diagnosis is in doubt.

Additional supportive evidence can be:

- Positive glycaemic (>1.5 mmol/L or 27 mg/dL) response to intramuscular glucagon [104].
- Positive glycaemic response to a subcutaneous dose of octreotide.
- Low serum concentrations of IGFBP1 (insulin negatively regulates the expression of IGFBP1) [105].

Management of Hyperinsulinaemic Hypoglycaemia

Prompt diagnosis and immediate management of HH is required to prevent severe brain damage and permanent neurodevelopmental disorders (Table 16.7). The aims of treatment are to avoid hypoglycaemia (generally defined as blood glucose of <63 mg/dL or <3.5 mmol/L), maintain normoglycaemia and restore ability of ketone body production since glucose and ketones provide the main and alternate energy requirements for the brain. Treatment includes medical, surgical or sometimes a combination of therapies.

CHI due to recessive mutations in *ABCC8/KCNJ11* genes is usually refractory to oral feeds and requires high concentrations of intravenous glucose to maintain normoglycaemia, but it may be possible to maintain normoglycaemia in the milder forms using oral feeds. Table 16.7 summarizes the treatment strategies used in the management of HH.

Medical Therapy

Diazoxide

Diazoxide is the first-line treatment for HH of all types and in all age groups (Table 16.8). Diazoxide binds to the SUR1 subunit of the K_{ATP} channel, which opens up and activates intact K_{ATP} channels, thereby reducing insulin secretion [110], but it works only in the presence of an intact K_{ATP} channel so children with diffuse disease due to inactivating mutations in *ABCC8* and *KCNJ11* and most patients with focal lesions are usually unresponsive.

Nifedipine

Nifedipine is a calcium channel blocker that inhibits insulin secretion by inactivation of voltage-gated calcium channels. Several child and adult patients with nifedipine-responsive forms of HH have been reported [111] but this drug has not been a reliable and sustainable treatment option for HH in clinical practice.

Octreotide

Octreotide is an analogue of somatostatin, which has potent inhibitory effects on the release of insulin from pancreatic β-cells. Somatostatin receptor 2 (SSTR2) is the predominant SSTR in the human islets having inhibitory effects on insulin secretion compared with mouse islets where SSTR5 mainly inhibits insulin secretion [112]. Somatostatin and its analogues can inhibit insulin secretion by activation of SSTR2 and SSTR5, which is mediated by stimulation of the Gi/Go protein [113]. In pancreatic β-cells, activation of SSTR inhibits calcium mobilization and acetylcholine activity and decreases insulin gene promoter activity resulting in reduced insulin biosynthesis [114, 115]. Although octreotide has an effect of rapidly and sharply increasing blood glucose with initial doses, tolerance to its effects in the form of tachyphylaxis may be observed after the subsequent 2–3 doses; this is generally transient and can be managed by dose adjustment.

Glucagon

In emergency situations like symptomatic hypoglycaemia, hypoglycaemic seizures and inability to gain venous access, intramuscular glucagon administration can be life-saving as it increases blood glucose within a few minutes. Glucagon immediately releases hepatic glucose stores by induction of glycogenolysis. Glucagon also stimulates gluconeogenesis, ketogenesis and lipolysis. It can be used alone or administered subcutaneously, or as an intravenous infusion, or used in combination with octreotide infusion in severe HH to achieve normoglycaemia. Some studies have reported using glucagon infusion up to 33 μg/kg/h [116] but high doses (>20 μg/kg/h) can stimulate insulin secretion and cause rebound hypoglycaemia [117].

Table 16.7 Summary of treatment for HH patients [92, 106, 107].

1) Emergency treatment	i) Glucose oral gel/glucose tablets or oral Glucojuice (glucose drink) ii) Frequent feeding (oral or intra-gastric) iii) Glucagon injection (intramuscular) iv) Intravenous bolus of glucose and maintenance infusion (may require central venous line to give >10% glucose infusion)
2) Medical management	i) Diazoxide ± chlorothiazide ii) Nifedipine iii) Glucagon infusion (intravenous or subcutaneous) iv) Octreotide infusion (subcutaneous) v) Acarbose (postprandial HH) vi) New medicines a) mTOR inhibitors (sirolimus, everolimus) b) Long-acting octreotide/long-acting somatostatin analogue (Lanreotide) c) Glucagon-like peptide 1 (GLP-1) antagonists – exendin (9-39)
3) Surgical management	Differentiation of histologic subtype by genetic analysis ± 18F-DOPA PET/CT scan i) Lesionectomy for focal disease (laparoscopy or laparotomy) or subtotal or near-total pancreatectomy for diffuse disease (laparoscopic or laparotomy) – removal of up to 95–98% of pancreas ii) Surgery for insulinoma (laparoscopic or open) – enucleation or resection of tumour iii) Postprandial hyperinsulinaemic hypoglycaemia (PPHH) – correction of gastric bypass that caused dumping syndrome iv) Surgical procedures that may help in management of hypoglycaemia – Nissen's fundoplication and percutaneous endoscopic insertion of gastrostomy (PEG)
4) Feeding management	i) High calorie and carbohydrate-rich feeds (e.g. maxijul or vitajul) – bolus or continuous intra-gastric feeding (based on fasting tolerance) ii) Use of cornstarch (usually given overnight) iii) Modification of diet/feeds if protein-sensitive or postprandial HH
5) Follow-up management	i) Assessment of neurodevelopmental outcome ii) Monitor height and weight iii) Blood glucose profile and fast tolerance – effect of medications and change of doses if required iv) Management of post-surgical complications (diabetes mellitus and exocrine pancreatic insufficiency) v) Imaging tests for recurring/relapsing malignant insulinomas

Acarbose

Acarbose is an inhibitor of the intestinal enzyme system, alpha glucosidase, which is involved in the hydrolysis of complex carbohydrates into glucose in the small intestine. Inhibition of intestinal glucosidase reduces the rate of digestion of complex carbohydrates to glucose molecules thereby decreasing glucose absorption. Although it is usually known as a drug for type 2 diabetes mellitus, in postprandial hypoglycaemia the net effect is the prevention of a rapid increase of postprandial blood glucose concentrations (postprandial hyperglycaemia), thereby reducing rapid release of insulin from pancreatic β-cells and subsequent hypoglycaemia [118].

Feeding

In some patients, frequent high volume, calorie- and glucose-enriched oral feeds and continuous enteral feeding contribute to the success of medical therapy. Children may require a gastrostomy and anti-reflux surgery to allow the delivery of frequent bolus and continuous overnight feeds. Long-term successful management with subcutaneous octreotide and glucagon injections/infusion in combination with frequent feeds has been reported.

Newer Medical Therapy in HH and Future Prospects

These medications have been reported to be effective in HH but further research is required before widespread

Table 16.8 Drugs used for medical therapy of hyperinsulinaemic hypoglycaemia [108, 109].

	Mode of action	Dose	Side effects
Conventional therapy (oral)			
Diazoxide (oral)	Binds to SUR1 subunit of K_{ATP} channels	5–20 mg/kg/day, in 3 divided doses	Fluid retention, oedema, hypertrichosis. Rarely can cause cardiac failure, pulmonary hypertension, blood dyscrasias such as anaemia/neutropenia, liver dysfunction and paradoxical hypoglycaemia if used in higher doses
Chlorothiazide (oral)	Synergistic effects with diazoxide on K_{ATP} channels and prevents fluid retention	7–10 mg/kg/day, in 2 divided doses	Hyponatraemia, hypokalaemia
Nifedipine (oral)	Inhibits Ca channels of the β-cell membrane	0.25–2.5 mg/kg/day, in 2–3 divided doses	Hypotension
Acarbose(oral) (sometimes used in PPHH)	Intestinal α-glucosidase inhibitor	75–300 mg/day divided into 3 doses. Usually given 5–10 minutes before feeds	Nausea, diarrhoea, abdominal cramps
Conventional therapy (bolus injections or infusion)			
Octreotide (s.c. bolus injections or infusion)	Activation of SSTR 5 inhibits calcium mobilization and acetylcholine activity	5–35 μg/kg/day, divided into 3–4 doses or continuous subcutaneous infusion	Nausea, vomiting, diarrhoea, abdominal discomfort, drug-induced hepatitis, long QT syndrome, tachyphylaxis, necrotizing enterocolitis in neonates. Long-term use can cause biliary sludge and gallstone formation and suppression of pituitary hormones (growth hormone, TSH)
Glucagon (s.c./i.m. bolus or s.c./i.v. infusion)	G-protein-coupled activation of adenylate cyclase, increases cAMP and promotes glycogenolysis and gluconeogenesis	0.02 mg/kg/dose or 2.5–10 μg/kg/h infusion	Nausea, vomiting. Rarely skin rash (necrolytic migratory erythema) and rebound hypoglycaemia in high doses (>20 μg/kg/h)
New therapies			
Rapamycin (sirolimus, everolimus) (oral)	mTOR inhibitor	No certain dose. Dose adjustment according to trough blood concentration, usually aim for between 5 and 15 ng/mL	Immune suppression causing sepsis, pneumonitis etc., mucositis, hyperlipidaemia, elevation of liver enzymes, thrombocytosis, impaired immune response to BCG vaccine
Octreotide LAR/ lanreotide (deep s.c. or i.m.)	Similar effects as daily multidose octreotide	Total 4 weekly dose of octreotide given 4 weekly or 15–60 mg 4 weekly	Similar to daily multiple injection of octreotide. However, long-term follow-up data unknown
Exendin (9-39) (i.v./ s.c. infusion)	GLP-1 receptor antagonist	0.02–0.1 mg/kg/h	Not determined

use is recommended, and currently they should be used with caution only in tertiary/quaternary referral centres.

mTOR Inhibitor (Sirolimus, Everolimus)

Sirolimus, also known as rapamycin, is an mTOR (mammalian target of rapamycin) inhibitor used in patients for prevention of allograft kidney rejection. It has been known to cause hyperglycaemia. The role of the mTOR pathway in β-cell overgrowth has been described in patients with diffuse CHI [119], and successful management of four patients with medically unresponsive CHI who were due to undergo pancreatectomy has been reported [120]. Sirolimus is administered orally and the starting dose is 0.5–1 mg/m^2/day in 2 divided doses. The dose is titrated by regular monitoring of sirolimus concentration. Children also need to be monitored for side effects, including potential immunosuppression causing frequent infections and rarely diabetes mellitus.

LAR-Octreotide/Lanreotide

Lanreotide, a synthetic octapeptide somatostatin analogue, has been widely used in adults for the treatment of acromegaly. It has high binding affinity for human somatostatin receptors (SSTR) 2 and 5 and reduced binding affinity for human SSTR 1, 3 and 4. Activity at SSTR 2 and 5 is the primary mechanism considered to be respon-

sible for GH inhibition [121]. Recently, two prolonged release formulations, LAR-octreotide and lanreotide, have been used successfully intramuscularly or by deep subcutaneous injection in a few patients with HH [122–124]. Four weekly injections have been shown to improve compliance and quality of life for patients and their families but more studies are required to assess the long-term effectiveness of this medication.

GLP-1 Receptor Antagonist Exendin (9-39)

GLP-1 is an insulinotropic hormone secreted from intestinal L cells after food ingestion. Postprandial HH seen in children with dumping has been linked to the effects of GLP-1. Blockade of GLP-1 by exendin (9-39), a GLP-1 receptor antagonist, has been evaluated as a potential therapeutic agent in patients with dumping syndrome and also in patients with K_{ATP} channel mutations [125]. Results have been promising, with elevation of fasting and postprandial blood glucose concentrations in small studies, but the drug needs further clinical experience on its effectiveness, safety and pharmacokinetics to be a therapeutic option in children with HH.

Surgical Therapy

Differentiation of the Histologic Subtypes of HH

Differentiation between diffuse and focal forms of HH is important for surgical management as focal disease can be cured by excision while medically unresponsive diffuse disease may require near-total pancreatectomy [102, 126, 127]. They are indistinguishable in terms of clinical presentation and biochemical features. Genetic analysis for mutations in *ABCC8/KCNJ11* combined with 18F-DOPA PET/CT scanning allows the differentiation of focal and diffuse disease with high sensitivity and specificity [107].

18F-DOPA PET/CT was first described as a non-invasive technique to diagnose focal hyperinsulinism of infancy [128]. Patients with a clear (18F) hotspot in the pancreas with maximum uptake (standardized uptake values) measuring >1.5 times the maximum pancreatic tracer concentration elsewhere were defined as having focal disease [129, 130]. The principle of this imaging technique is based on the fact that pancreatic islets take up L-DOPA and convert it into dopamine using the enzyme DOPA decarboxylase, which is expressed in islet cells [131]. 18F-DOPA is an analogue of DOPA and thus the positron-emitting compound is useful for tracking the uptake of this dopamine precursor. Both diffuse and focal diseases have a high DOPA decarboxylase activity.

Surgical Management of HH

Surgery of Focal Disease Localization of focal lesions with subsequent partial pancreatectomy leads to cure in most patients without the post-operative complications of diabetes mellitus and pancreatic exocrine insufficiency. Complete excision of the focal lesion requires intraoperative biopsies to look for abnormal cells at the margin. To ensure complete resection and avoid repeat surgery, additional resections may be needed until margins are clear. For large focal lesions in the pancreatic head not amenable to local resection, pancreatic head resection with Roux-en-Y pancreatico-jejunostomy is a safe and effective procedure [132], but there are also reports of a patient with a focal lesion in the head of the pancreas who underwent pancreatic head resection preserving the main pancreatic duct to avoid pancreatico-jejunostomy [133].

Surgery for diffuse disease Medically unresponsive diffuse CHI requires 95–98% pancreatectomy but up to 50% patients continue to have hypoglycaemia or they develop post-operative diabetes mellitus and exocrine pancreatic insufficiency [134, 135]. Laparoscopic pancreatectomy (partial or near total) represents a new approach to the management of infants with CHI [136] reducing hospital stay and post-operative complications.

Hypoketotic Hypoinsulinaemic Hypoglycaemia

AKT2 mutations

AKT2, a serine/threonine kinase, is highly expressed in insulin-sensitive tissues and required for the insulin-induced translocation of GLUT4 to the plasma membrane [137, 138]. A missense p.R274H loss-of-function mutation in *AKT2* resulted in severe insulin resistance, marked hyperinsulinaemia, and diabetes mellitus [139]. However, the opposite phenotype caused by activating *AKT2* mutations leads to hypoinsulinaemic hypoglycaemia [140]. Recently MORFAN (mental retardation, pre- and postnatal overgrowth, remarkable face, and acanthosis nigricans) syndrome has been reported due to a *de novo AKT2* mutation leading to hypoinsulinaemic hypoglycaemia [141].

Abnormal Processing of IGF-II

Persistent hypoketotic, hypofattyacidaemic hypoinsulinaemic hypoglycaemia can occur in very rare circumstances as a result of non-islet cell tumour hypoglycaemia (NICTH) [142]. In this condition, large amounts of incompletely processed, high molecular weight IGF-II precursor proteins ('big' proIGF-II) are produced by neoplastic cells and have insulin-like activity in the body [143].

Factitious Hypoglycaemia

Factitious hypoglycaemia can be induced pharmacologically, intentionally as a diagnostic tool, accidentally as a

complication of the treatment of diabetes mellitus or as a consequence of poisoning either with insulin itself or with drugs such as sulphonylureas [144], which stimulate insulin release. The biochemistry will be a raised insulin concentration with normal C-peptide (insulin/c-peptide ratio of >1) in the case of insulin administration.

Failure of Counter-Regulatory Hormones

Deficiency of counter-regulatory hormones can cause hypoglycaemia. Deficiency of glucagon or epinephrine, the two hormones important for the immediate restoration of the blood glucose concentration, is rare. There has been a single case report describing glucagon deficiency [145]. However, the biochemistry seems to be suggestive of congenital hyperinsulinism and it is now known that the serum glucagon counter-regulatory hormonal response to hypoglycaemia is blunted in congenital hyperinsulinism [146]. In the same study, it was found that children with CHI had an appropriate rise in epinephrine and norepinephrine concentrations in spite of a blunted glucagon response. The possible mechanisms for blunted glucagon response could be due to the prolonged suppressive effect of hyperinsulinism on the α-cell.

More common is hypoglycaemia brought about by GH and/or cortisol deficiency that results from reduced gluconeogenic substrate availability (decreased mobilization of fats and proteins) and increased glucose utilization because of increased insulin sensitivity of tissues in the absence of GH and cortisol. The threshold for the release of GH and cortisol in adults lies within or just below the physiological blood glucose concentration, implying that GH and cortisol start to rise in response to blood glucose concentrations within the normoglycaemic range and that these increases are probably inversely proportional to the nadir in blood glucose [147]. GH and cortisol respond differently to spontaneous hypoglycaemia compared with that induced by insulin infusion (insulin tolerance test) [148]. This may be related to the rate of fall of the blood glucose concentration, which is why a low GH value at the time of spontaneous hypoglycaemia may not necessarily indicate GH deficiency.

Pituitary Disorders

GH and ACTH deficiencies are seen in congenital or acquired disorders of pituitary function. Congenital hypopituitarism may present with life-threatening hypoglycaemia, abnormal serum sodium concentration, shock, conjugated hyperbilirubinaemia, microphallus and growth failure. The incidence of hypoglycaemia in panhypopituitarism can be as high as 20% and hypoglycaemia associated with hypopituitarism may be a cause of sudden death [149]. Standard replacement doses of hydrocortisone and GH prevent hypoglycaemia, although the dose of hydrocortisone will need to be increased during stress. Congenital hypopituitarism may be the result of complications around delivery or due to hypoplasia of the gland, sometimes associated with specific genetic abnormalities such as mutations in *POU1F1*, *PROP1*, *LHX3*, *LHX4*, *SOX3*, *OTX2*, *GLI2* and *HESX1*. Bardet–Biedl and Prader–Willi syndromes have also been associated with pituitary hormone deficiencies. Acquired hypopituitarism may result from tumours (most commonly craniopharyngioma), radiation, infection, hydrocephalus, vascular anomalies and trauma (Chapter 5).

Adrenal Disorders

The common symptoms of adrenal insufficiency are fatigue, light-headedness upon standing, muscle weakness, fever, weight loss, anxiety, nausea, vomiting, diarrhoea, headache, sweating, changes in mood or personality and joint and muscle pains. Some patients have cravings for salt or salty foods due to the loss of sodium through their urine. Adrenal disorders should be considered where the patient describes a recent increase in pigmentation and are confirmed by the presence of low cortisol concentrations with a significantly increased ACTH concentration. Skin changes are not encountered in secondary and tertiary hypoadrenalism. Hyperinsulinaemic infants may mount a suboptimal cortisol response to hypoglycaemia, which can cause difficulty in interpretation; a short Synacthen test with basal ACTH concentration can be useful in these circumstances. Adrenal disorders can be classified into adrenal dysgenesis, impaired steroidogenesis and adrenal destruction [150] (Chapter 9).

Adrenal Dysgenesis or Hypoplasia

The clinical forms of adrenal dysgenesis or hypoplasia may occur secondary to impairment of the hypothalamic–pituitary axis or as a part of an ACTH resistance syndrome or as a primary adrenal defect due to mutations in key genes in adrenal development (including mutations in *NR5A1* encoding SF1, *NROB1* encoding DAX-1, *CDKN1C* causing IMAGe syndrome, *SAMD9* causing MIRAGE syndrome and an autosomal recessive form due to an unknown aetiology). Adrenal hypoplasia may also be associated with rare

syndromes such as Pallister–Hall syndrome, Meckel–Gruber syndrome, Pena-Shokeir syndrome and Galloway-Mowat syndrome. Deficiency of steroidogenic factor 1 (SF-1) on chromosome 9q33 can lead to adrenal failure with complete XY sex reversal due to testicular dysgenesis. In females, the ovaries may be spared, although *SF1* mutations can be associated with premature ovarian failure. Congenital X-linked adrenal hypoplasia is caused by mutations in the dosage-sensitive sex reversal adrenal hypoplasia gene 1 (*DAX-1*), which can present in males in the first few months of life as the fetal adrenal cortex atrophies. The gene on Xp21.3 encodes a nuclear hormone receptor and expression of DAX-1 is important for development of the gonads, adrenal cortex, hypothalamus and pituitary [151]. The condition is associated with hypogonadotropic hypogonadism. Loss of this gene may be part of a contiguous gene syndrome with DAX-1, glycerol kinase deficiency and occasionally Duchenne muscular dystrophy. Familial glucocorticoid deficiency (FGD) is a rare autosomal recessive syndrome characterized by a failure of cortisol production because of adrenal ACTH resistance. Mutations in several genes have been described in association with FGD, and these are covered in more detail in Chapter 9. AAA syndrome occurs in males and females and should be considered when glucocorticoid deficiency occurs with other features including achalasia, alacrima and autonomic neuropathy in association with hyperpigmentation.

Impaired Steroidogenesis

Congenital adrenal hyperplasia (CAH) caused by 21-hydroxylase deficiency is one of the most common autosomal recessive disorders in humans. 21-Hydroxylase is encoded by *CYP21A2* and the estimated carrier frequency of deleterious CYP21 mutations is 1 in 50. The CAH phenotype reflects the degree of 21-hydroxylase enzyme deficiency. Complete enzyme deficiency, with impairment of both cortisol and aldosterone synthesis, results in the salt-wasting form characterized by prenatal virilization in females and salt-wasting crises in the neonatal period. Partial enzyme deficiency leads to simple virilizing CAH characterized only by prenatal virilization in females and pseudo-precocious puberty in males and females. The incidence of CAH is 1 in 15,000 live births. The first clue to the condition in a male infant may be collapse in the first 1–8 weeks of life with hypoglycaemia, hypotension and hyperkalaemia [152]. Other causes of impaired steroidogenesis are much less common.

Adrenal Destruction

Primary adrenal failure is of autoimmune origin in ~50% of cases and can be associated with failure of other endocrine organs in the polyglandular syndromes. Adrenoleukodystrophy can be confirmed in males by measurement of plasma long chain fatty acids and can lead to hypoglycaemia [153]. Adrenal destruction from haemorrhage or ischaemia can occur in the context of a severe systemic illness, such as neonatal hypoxia or meningococcal septicaemia.

Inborn Errors of Metabolism Presenting with Hypoglycaemia

Disorders of Glycogen Synthesis and Breakdown

Glycogen is a large complex molecule formed in the fed state and stored abundantly in liver and muscle tissue. Glycogen breakdown to glucose is essential for the maintenance of normoglycaemia, becoming important ~4 hours following a meal, although this varies according to age (Figure 16.4).

Several inherited abnormalities in glycogen degradation, synthesis and glucose release cause disease. Collectively they are known as glycogen storage diseases (GSD) or glycogenoses. Clinical presentation may be conveniently considered in two phenotypes, hepatic and muscular, although different pathophysiological mechanisms underlie each disorder. Within the scope of this chapter hepatic GSD causing hypoglycaemia will be considered. For a more complete review see Ref. [154].

Glycogen Storage Disease Type Ia [Von Gierke Disease, Glucose-6-Phosphatase Deficiency, *G6PC* Gene]; Glycogen Storage Disease Type Ib [Glucose-6-Phosphate Transporter, *SLC37A4* Gene]

This disorder typically presents in early infancy (4–6 months) or sometimes in the neonatal period. Hypoglycaemia is a prominent feature and patients usually have a very short fasting tolerance with severe hypoglycaemia developing 3–4 hours after feeding. The history will often be of a child who feeds frequently and in whom symptoms of sweating and irritability are often present. The first presentation to medical services may be with hypoglycaemic seizures following an overnight fast. Examination will reveal an abdomen distended with hepatomegaly (present from 4 weeks), doll-like facies and faltering growth. In addition to the above, patients with GSD Ib also may have recurrent infection due to neutropenia and leucocyte dysfunction and symptoms of inflammatory bowel disease.

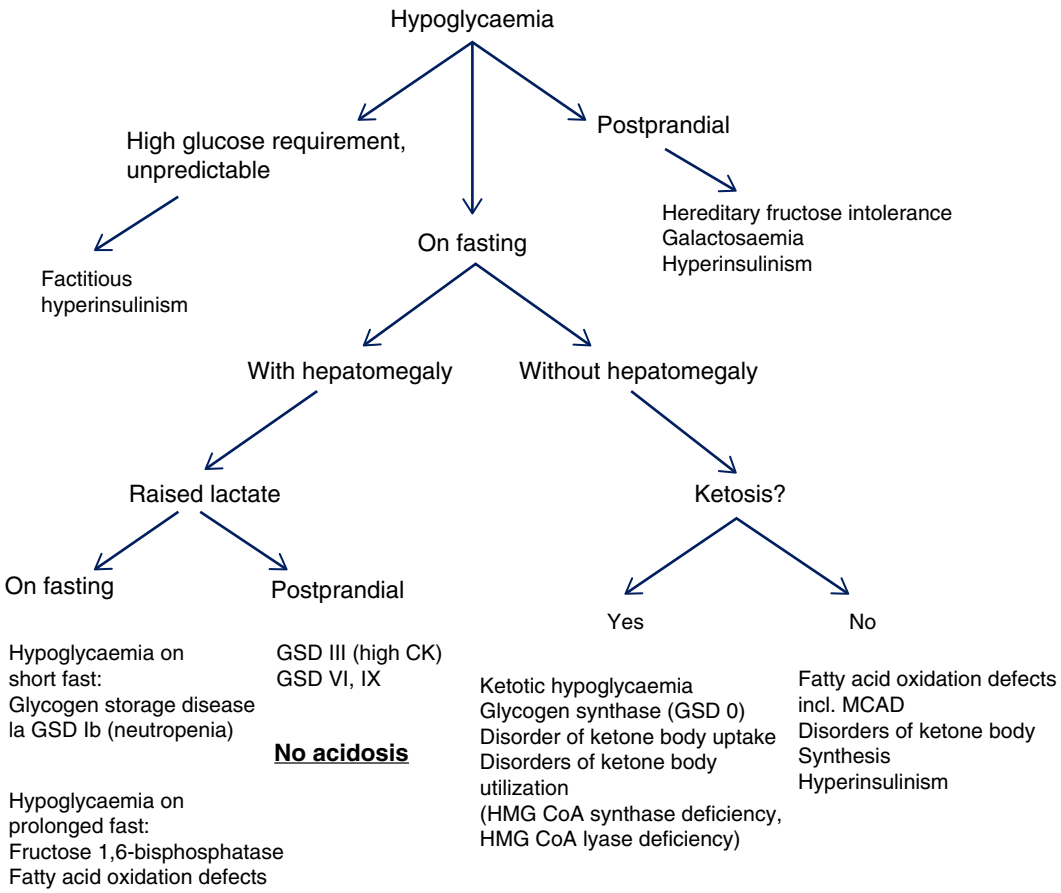

Figure 16.4 Diagnostic workup for hypoglycaemia.

The genetic deficiency of the glucose-6-phosphatase enzyme (GSD Ia) or its microsomal transport system (GSD Ib) results in a twofold detrimental effect on glucose metabolism; the enzyme is required as the final step for glucose release from hepatic glycogen and is also essential for production of glucose via gluconeogenic pathways. Thus glucose production is impaired from both glycogenolysis and gluconeogenesis. A secondary cascade of metabolic derangement is also present in GSD I.

Glycogen Storage Disease Type III [Debrancher, Amylo-1,6-Glucosidase Deficiency *AGL* Gene]

Deficiency of the debrancher enzyme results in excess storage of glycogen of abnormal structure in liver and muscle tissue. This diagnosis is usually made in early childhood and clinically GSD III appears very similar to GSD I with characteristic hypoglycaemia, hepatomegaly, hyperlipidaemia and growth failure. Splenomegaly and nephromegaly may be evident on examination. Skeletal myopathy and cardiomyopathy tend to develop over time and are more prominent in older children and adults.

In contrast to GSD I, fasting ketosis, elevated CK and elevated liver transaminases may feature in GSD III. Although these are useful biochemical clues, a firm diagnosis is made using molecular genetic techniques and enzyme activity in lymphocytes may also be useful.

Management of children with GSD III tends to be more straightforward than in GSD I, although the principles are the same. Regular daytime feeding and continuous overnight feeds usually requiring a feeding tube (nasogastric or gastrostomy) are the mainstay.

General Approach to the Patient with Suspected GSD

Laboratory Investigation Fasting hypoglycaemia in the presence of elevated lactate is the hallmark of GSD I. Secondary biochemical changes will also be evident with severe lipaemia, hypertriglyceridemia, elevated CK (GSD III) and elevated uric acid. A definitive diagnosis should be made with molecular genetics.

Clinical Management Management is aimed at maintenance of normoglycaemia, prevention of secondary metabolic consequences and promotion of normal growth.

This involves provision of frequent feeds, usually via nasogastric tube in early years and by continuous infusion overnight. Later in childhood slowly resorbed carbohydrates such as uncooked cornstarch may be used. Careful management during illness is crucial to avoid devastating hypoglycaemia. Patients are provided with an 'emergency regimen' (ER) for use during illness, which is adjusted with increasing age and weight. This ER is used continuously during periods of illness and consists of frequent glucose polymer drinks. Where this is not tolerated, an intravenous infusion of dextrose should be provided to deliver up to 8 mg/kg/min glucose (see www.BIMDG.org).

The neutropenia of GSD Ib may necessitate granulocyte colony-stimulating factor (G-CSF). In some cases liver transplantation, which has been shown to be of good effect in some patients, may be considered.

Glycogen Storage Disease 0, Glycogen Synthase Deficiency

This disorder is not strictly a GSD as the enzyme deficiency in fact leads to a deficiency of glycogen [155]. Absence or reduced activity of glycogen synthase results in reduced amounts of stored glycogen in the liver and hypoglycaemia in the fasted state as a result. Affected patients usually present in infancy with early morning fatigue and sometimes hypoglycaemic seizures with hyperketonaemia, but they do not have hepatomegaly or hyperlipidaemia. A clue to diagnosis is transient postprandial hyperglycaemia and elevated lactate, which probably reflects glucose conversion to lactate in the absence of glycogen synthesis. Diagnosis is usually made using molecular genetic techniques.

Management is symptomatic and aimed at avoidance of fasting. Patients have regular, protein-rich meals throughout the day and a complex carbohydrate such as uncooked cornstarch is required overnight to prevent morning hypoglycaemia.

Disorders of Mitochondrial Fatty Acid Oxidation and Ketone Body Synthesis

During fasting, the major source of fuel for cells when circulating glucose and glycogen stores are depleted becomes oxidation of fatty acids from adipose tissue. Inborn errors of metabolism that affect these pathways tend to present after periods of prolonged fasting, during intercurrent illness or during the newborn period, especially if feeding is not well established.

Fatty acids are used by heart muscle in preference to glucose and are the main fuel for skeletal muscle during sustained exercise. In addition to releasing energy, hepatic fatty acid oxidation provides acetyl-CoA for ketone body synthesis. By using ketone bodies, the brain can also derive energy indirectly from fatty acids.

Mitochondrial β-oxidation of fatty acids is a complex pathway and several inherited defects have been described. Circulating fatty acids are released from triglyceride, activated to an esterified CoA species, and enter the cell. As the mitochondrial membrane is impermeable to long chain fatty acids, they enter the mitochondrial matrix via the carnitine transporter where sequential shortening of the fatty acid through a series of enzyme reactions, progressing from very long chain fats through to short chain fats, takes place. Each step generates energy in the form of electrons, which enter the electron transport chain. Ultimately acetyl-CoA is formed, which is converted in the liver to ketone bodies, which may be used directly by the brain as a source of energy and are also transported from the liver to other tissues as a source of energy. Individual disorders are named according to the particular enzyme deficiency; others affect the pathway in a more general way (see Table 16.1).

Deficiency of medium chain acyl-CoA dehydrogenase (MCADD) warrants particular mention as it is the most common of the fatty acid oxidation disorders (estimated incidence in the UK is 1 in 10,000) and it has been on the UK newborn screening programme since 2009. This condition highlights many of the typical features of the 'hepatic phenotype' of β-oxidation where patients present with episodic hypoketotic hypoglycaemia provoked by fasting and/or illness. Although these episodes may be life threatening and before newborn screening were often fatal, children are entirely normal and asymptomatic between episodes [156].

As fatty acids are a particularly important form of energy in muscle, some patients present with skeletal and/or cardiac muscle weakness. Examples include very long chain acyl-CoA dehydrogenase deficiency (VLACDD).

The clinical spectrum of a 'muscle phenotype' is varied and children may present with myopathy, muscle pain, rhabdomyolysis or relatively mild exercise intolerance. In some patients, life-threatening cardiomyopathy is the main feature. Biochemical derangement may not be pronounced so hypoglycaemia is not always a feature in these patients [154].

General Approach to the Patient with a Suspected Fatty Acid Oxidation Disorder

The biochemical hallmark of fatty acid oxidation disorders is 'hypoketotic hypoglycaemia'. It should be borne in mind however that ketones may be present in some cases, although the amount is inappropriately low for the clinical condition. Other biochemical derangements include lactic acidosis, liver dysfunction, elevated creatine kinase and moderate hyperammonaemia. Hypoglycaemia is usually a late sign of decompensation and should be acted upon as a matter of urgency.

Accumulation of fatty acid intermediates may be detected in blood and urine. Specific acylcarnitine profiles will often aid diagnosis. In some instances, where diagnosis is not straightforward, dynamic testing of fatty acid flux in patient fibroblasts may be required. Molecular genetic confirmation should also be sought.

The mainstay of management for disorders of fatty acid oxidation is avoidance of fasting. In this way the body is never required to use fat stores to generate energy and the cycle of decompensation is avoided. To achieve this, carbohydrate is provided as an alternative fuel, either orally as a drink or intravenously where necessary.

In practical terms this requires that patients are provided with an 'ER' adjusted for age and weight, which is used continuously during periods of illness and consists of frequent glucose polymer drinks to prevent fat catabolism. In some instances of severe illness or the inability to tolerate enteral fluids, intravenous 10% dextrose with electrolytes at a rate to provide glucose delivery 6–8 mg/kg/min may be needed (see www.BIMDG.org.uk). Children should also be managed cautiously during periods of fasting for general anaesthesia and dextrose containing intravenous fluids provided. Hyperglycaemia should be managed with low-dose insulin infusion to maintain anabolism and glucose delivery should not be reduced.

Some patients with severe defects cannot safely tolerate an overnight fast, thus have continuous overnight feeds or use a complex carbohydrate source such as uncooked cornstarch.

Those that experience severe pain and possibly rhabdomyolysis (long and very long chain defects) with exercise will often use a carbohydrate-rich snack or feed beforehand to reduce symptoms.

Idiopathic Ketotic Hypoglycaemia (IKH)

IKH is the most common cause of hypoglycaemia in 1- to 5-year-olds and is a diagnosis of exclusion, which should be made only when other causes of hypoglycaemia have been excluded. Disorders that have been misdiagnosed as IKH and that lead to hypoglycaemia in the presence of ketonaemia include GSD IX, GSD 0, GH deficiency, adrenal insufficiency and fructose-1,6-bisphosphatase deficiency and thorough investigation must precede a diagnosis of IKH.

There is no known enzyme defect in IKH, although it is possible that the diagnosis is yet to be made in some cases. It is probable that IKH represents the lower tail of the Gaussian distribution of fasting tolerance in children [157] and is sometimes termed 'accelerated fasting'.

Children with IKH present between 1 and 8 years with hypoglycaemia in the presence of a good ketone response, which may be measured in blood or urine. Hypoglycaemia is usually provoked by intercurrent illness and there is often a history of poor intake and vomiting. It is not uncommon for the child to experience hypoglycaemic seizures in the context of IKH but it is a benign condition that tends to improve spontaneously beyond 8 years of age [154].

General Approach to the Patient with Idiopathic Ketotic Hypoglycaemia

A comprehensive 'hypoglycaemia screen' (Tables 16.2 and 16.3) will exclude recognized metabolic and endocrine causes of ketotic hypoglycaemia, but a fast provocation test may be required in some cases.

The mainstay of management is to prevent hypoglycaemia with a high calorie 'ER' feed (glucose polymer drink) during periods of illness or fasting. If this is not tolerated, patients are advised to attend hospital where intravenous dextrose may be required. Some brittle patients may require complex carbohydrate such as uncooked cornstarch overnight to prevent morning hypoglycaemia but the majority can fast overnight easily when well. It is generally advised that patients do not fast beyond 12 hours.

Disorders of Gluconeogenesis

Gluconeogenesis pathways form glucose to maintain normoglycaemia from non-carbon skeletons and this process is required during fasting. It is not simply a reversal of glycolysis because some steps require specific enzymes to overcome reversible reactions on the pathway.

Children with inborn metabolic defects of the gluconeogenesis pathways typically present with recurrent episodes of hypoglycaemia in the presence of lactic acidosis. Ketosis is variable. The two most common causes are deficiency of fructose-1,6-bisphosphatase and deficiency of glucose-6-phosphatase (GSD I) [153].

Deficiency of fructose-1,6-bisphosphatase, a key enzyme on the gluconeogenic pathway, impairs glucose production from all gluconeogenic precursors, including fructose. It leads to hypoglycaemia with accumulation of gluconeogenic substrates (lactate, glycerol) when stores of glycogen are depleted during periods of fasting or in the newborn period [158]. Children present in early childhood, and ~50% will do so in the first days of life.

Typically a child will present with respiratory distress and hyperventilation due to lactic acidosis and hypoglycaemia. Hepatomegaly is often present but liver function tests (transaminases) are normal. The child may deteriorate rapidly without prompt intervention with hypotonia

progressing to coma and death. It is not unusual for patients to have several such episodes before a diagnosis is made. As in many inborn errors, periods of fasting or catabolism due to illness are the trigger for decompensation and patients are well with normoglycaemia and normal acid–base status between episodes.

Large amounts of fructose (ingested or intravenous) given after fasting or during illness may provoke decompensation that may be lethal. Chronic ingestion of fructose between acute attacks when the child is well does not have a detrimental effect [154].

General Approach to the Patient with Fructose-1,6-Bisphosphatase Deficiency

Hypoglycaemia and elevated lactate are usually but not always accompanied by ketonaemia and ketonuria. Characteristic metabolites such as lactate, pyruvate, ketone bodies and 2-oxoglutaric acid may be seen in a urinary organic acid profile. Plasma amino acids show elevated alanine. A definitive diagnosis should be made on white cell enzyme assay of fructose-1,6-bisphosphatase and confirmed with molecular genetics (*FBP1* gene).

The acute metabolic derangement usually responds rapidly to treatment with oral or intravenous glucose. Sodium bicarbonate may be required in the short term to correct acidosis but is not needed as regular maintenance.

Prolonged fasts should be avoided and some children require overnight feeds or uncooked cornstarch if they cannot tolerate an overnight fast. Illness should be managed promptly with use of an oral glucose polymer 'ER'. If this is not tolerated, intravenous dextrose should be administered.

Fanconi Bickel Disease, GLUT2 Transporter Deficiency (*GLUT2*)

Hypoglycaemia may be the presenting feature of Fanconi Bickel syndrome (FBS), which is caused by deficiency of a glucose and galactose transporter (GLUT2) present on hepatocytes and the basolateral membranes of the renal proximal tubule and enterocyte [159]. It is inherited in an autosomal recessive manner. Patients present between 3 and 10 months of age with hepatomegaly due to glycogen accumulation, which develops over time. Nephromegaly may be seen on ultrasound. There is a tendency to hypoglycaemia in the fasted state and an intolerance to glucose and galactose in the fed state, which may be detected on 24 hour glucose profile or glucose load. A Fanconi-type nephropathy is a prominent feature leading to glycosuria and hypophosphataemic rickets. Failure to thrive is common; neurodevelopment is within normal limits [154].

General Approach to the Patient with Fanconi Bickel Syndrome

In the fed state, postprandial hyperglycaemia is evident due to impaired liver uptake of glucose. In contrast, during fasting when extracellular glucose falls, glucose and glucose-6-phosphate levels in hepatocytes are inappropriately high, which stimulates glycogen synthesis and inhibits gluconeogenesis and glycogenolysis causing hypoglycaemia. Urinalysis reveals glycosuria, phosphate and bicarbonate loss. The large loss of glucose in urine also contributes to the hypoglycaemia of FBS.

Treatment is symptomatic with a diet adequate in calories and frequency often with slowly absorbed carbohydrates to compensate for renal losses. The biochemical derangement secondary to the renal tubulopathy should be addressed and supplements of phosphate, bicarbonate and vitamin D are usually required.

Diagnosis is achieved through the typical biochemical findings and detection of mutations in the *GLUT2* gene.

Liver Disease

Acute liver failure of any aetiology may cause fasting hypoglycaemia due to glycogen depletion and impaired gluconeogenesis. Some inborn metabolic defects that are particularly associated with liver disease, such as tyrosinaemia type I, mitochondrial respiratory chain disease and galactosaemia, may therefore be associated with hypoglycaemia [160].

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17

Obesity

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KEY LEARNING POINTS

- Differential diagnosis of obesity
- Aetiology of obesity
- Genetic background of obesity
- Neurobiology of satiety and hunger
- Biology of adipose tissue
- The role of nutrition and malnutrition in obesity
- Epidemiology of obesity in developed/developing countries
- Co-morbidities of obesity
- Multidisciplinary therapeutic approach in obesity

Introduction

The prevalence of overweight and obesity in children and adolescents across most developed and developing countries is 20–35%. Although this seems to stabilize or even slightly decline in some countries, the number of adolescents and adults with obesity is still increasing dramatically worldwide. This is a major concern considering the well-described association of overweight and obesity with long-term health problems, such as cardiovascular disease (leading, for example, to myocardial infarction and stroke), type 2 diabetes and cancer.

Weight gain in the majority of individuals is the result of exposure to an 'obesogenic' environment superimposed on a background of genetic susceptibility brought about through evolutionary and cultural adaptation (Table 17.1). Approximately 40–70% of inter-individual differences in body weight and fat content are thought to be due to genetic variation and hence biological diversity. A large number of genes have been identified by genome-wide association studies (GWAS) and candidate gene approaches that are associated with the regulation of appetite, food intake and body weight. Therapeutic and preventive strategies have to follow complex and multifactorial themes and are often neither efficient nor effective.

Definitions, Differential Diagnosis, Assessments and Measurements

The definition and diagnosis of obesity in children and adolescents is surprisingly difficult and controversial. The level of fatness at which morbidity and mortality increases is determined on an actuarial basis. Body fat mass in children and adolescents depends upon ethnic and genetic background, gender, developmental stage and age. Waist and neck circumference, skinfold thickness and body mass index (BMI) are the most useful non-invasive clinical measures to determine the degree of body fatness and define obesity. Waist circumference (WC) and waist-to-hip ratio are helpful in assessing upper body fat deposition but do not measure intra-abdominal fat accumulation or accumulation of fat in other organs.

Limited normative data are available for some populations in respect to WC but more comprehensive data are available for skinfold thicknesses. BMI has been used as a surrogate marker of body fat and is the tool most frequently used to diagnose overweight and obesity at a young age, but BMI has surprisingly low sensitivity and specificity when compared with other body mass indices such as skinfold measurements and WC. Comparative data and reference values related to ethnicity, gender, pubertal stage and age have to be used and centiles or standard deviation scores have to be

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Companion website: www.wiley.com/go/dattani/brookcpe7

calculated to give a good representation of a subject's fat and lean body mass.

The relative unreliability of BMI for diagnosing childhood obesity is partly due to the fact that, for example, in early puberty in boys, body mass increments are due to muscle and bone mass increase, while in girls there are also adipose tissue increments in stages of early puberty. The clinical importance of determining visceral obesity and intra-organ fat deposits needs to be emphasized and WC, mid-upper arm circumference and neck circumference do reflect visceral and organ adipose tissue and are related reliably to most of the co-morbidities in obese subjects (Figure 17.1). Bioimpedance (BIA) or DEXA methodologies are sometimes used in clinical research to determine or monitor body fat but BIA may be unreliable and DEXA is considered invasive, so neither method should be used in routine clinical practice.

Direct measurements of body fat content (hydrodensitometry, BIA or DEXA) are interesting but BMI (weight in kilograms divided by the square of the height in metres) is easy to calculate and relates adequately to the direct measures, so BMI is frequently used to define obesity. In adults, a BMI >28 kg/m^2 is associated with an increased risk of morbidity from stroke, ischaemic heart disease, sleep apnoea syndrome, orthopaedic diseases or type II diabetes mellitus (DM). BMI between 25 and 29.9 kg/m^2 is grade 1, >30 kg/m^2 is grade 2 and >40 kg/m^2 grade 3 overweight (WHO classification). A central distribution of body fat is associated with a higher risk of morbidity and mortality, and an increased risk of death from cardiovascular disease and myocardial infarction and stroke has been found in adults whose BMI had been >75th centile in adolescence; hence childhood obesity increases the risk of subsequent morbidity and mortality whether obesity persists into adulthood or not.

A working definition of overweight in childhood has previously been a BMI > 85th centile and morbid or severe obesity has occasionally been defined by a BMI > 99th centile for age and gender. The current operational definition of childhood obesity should be

Table 17.1 Factors that contribute to the development of obesity and may constitute risk factors for a child to develop increased body weight and fat mass.

Genetic factors:
Possibly polymorphisms and/or mutations in any of the following: Adrenergic receptors, leptin, Ob-R, SOCS-3, TNF, POMC, MCH, MC4R, NPY, NPY receptors, CRH, TRH, urocortin, orexin A and B, galanin, neurotensin, serotonin and many others
Polygenic causes
Environmental/exogenous factors:
Increase of sedentary activities (screen time etc.)
Decrease in physical activity
Shift in diet towards more fast/pre-packaged foods with high fat/calories
Content
Sugar-sweetened beverages
Endocrine-disrupting chemicals
Loneliness and social isolation
Psychosocial/family problems
Low parental income
Low parental education

Figure 17.1 The calories overflow hypothesis. Surplus energy consumption leads to an overflow of energy in terms of fat to subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT) and finally to other organs such as the liver, heart or pancreas.

Table 17.2 Disorders that can present with obesity in childhood – differential diagnosis of obesity disorders.

Endocrine disorders such as:
Cushing syndrome/disease
Hypothyroidism
Growth hormone deficiency
Hyperinsulinaemia
Pseudohypoparathyroidism (Albright's hereditary osteodystrophy)
Central nervous system disorders/brain damage in relation to:
Hypothalamic tumour
Neurosurgery
Trauma
Post-inflammation (meningoencephalitis)
Post-chemotherapy
Corticosteroid therapy
Anti-epileptic therapy
CNS irradiation

that a child with BMI > 90th but <97th centile is overweight and a BMI > 97th centile is classified obese (European Task Force for Childhood Obesity). The International Obesity Task Force has proposed that the corresponding adult BMI cut-off points (25 and 30 kg/m^2) should be linked to BMI centiles in children to provide child cut-off points and age- and gender-specific BMI values from 2 to 18 years. These cut-off points are less arbitrary and less confined to specific populations and should provide internationally comparable prevalence rates of overweight and obesity in children.

The differential diagnosis of obesity can be difficult. 'Exogenous obesity', which may also be referred to as 'simple' or 'primary' obesity, is still the most common diagnosis but in addition to many monogenic traits of morbid obesity, there are many rare disorders that present with obesity early in life. These include genetic syndromes and a variety of underlying disorders such as hypothalamic tumours, other brain lesions and endocrine disorders (Table 17.2; see also Chapter 18). The diagnosis of primary or simple or 'exogenous' obesity is usually easy from family and personal history and a careful physical examination and more extensive laboratory tests and molecular genetic analysis will rarely be needed in routine clinical practice. No laboratory investigations are recommended unless substantial co-morbidity such as hypercholesterolemia or liver disease is suspected but a primary determination of serum concentrations of thyroid-stimulating hormone (TSH), free T3 and T4 and lipid measurements are carried out in most centres in Europe and the USA.

Aetiology of Obesity

The origins of obesity derive from an interplay between many genes, epigenetics, adipose tissue factors (inflammatory molecules, adipocytokines and immune cells), signalling molecules, food ingredients, metabolites, microbiota and environmental chemicals. In addition, social inheritance, obesogenic environment, urbanization and sociodemographic factors (income, poverty and education) play an important role in the development of obesity both in individuals and in societies. Nutrition and malnutrition, lack of physical activity and sedentary behaviour, media use and cultural habits and beliefs add to the obesogenic risks (Table 17.1, Figures 17.2 & 17.3). Lastly, human evolution has led to an increased prevalence of obesity in the recent history of mankind.

Monogenic forms of obesity are rare but are present in a significant cohort of patients affected by extreme obesity of early onset. It is important to diagnose children with the monogenic obesity forms *since treatments* are available for some of these conditions, and counselling the family as well as making appropriate decisions for care and schooling must be based upon clear diagnoses. Most genes identified in monogenic cases of obesity (genes encoding for the melanocortin 4 receptor [*MC4R*], pro-opiomelanocortin [*POMC*] or leptin and the leptin receptor) appear to be involved in the central regulation of energy intake. Variants of genes involved in energy utilization (those encoding β-adrenergic receptors 2 and 3, hormone-sensitive lipase and mitochondrial uncoupling proteins 1, 2 and 3) have also been associated with common obesity. Of the single gene defects that lead to obesity, *MC4R* defects are relatively common, accounting for ~4% of cases of early-onset childhood obesity. Large chromosomal deletions and copy number variations have also been linked to early-onset obesity. Large deletions of chromosome 16p11.2 are, for example, identified in ~0.7% of patients with morbid obesity.

Syndromic obesity (Table 17.3): A number of syndromic disorders are associated with severe and mostly early-onset obesity. They usually include mild to moderate mental retardation and other clinical features. Many are caused by alterations of the neuroendocrine signalling circuits that regulate hunger and satiety either by disturbing ciliary function in CNS neurons (e.g. Bardet–Biedl syndrome) or interfering with hypothalamic function (e.g. Prader–Willi syndrome). Table 17.3 gives an overview on the most frequently occurring forms of syndromic obesity.

Polygenic factors (Table 17.1) are related to the high incidence of childhood obesity. Genetic/endogenous and environmental/exogenous factors contribute to the development of a high degree of body fatness early in life and twin studies suggest that at least 50% of the tendency towards obesity is inherited. There is increasing evidence that responsiveness to dietary intervention is genetically determined.

Approximately 40–70% of inter-individual differences in body weight and composition are thought to be due to genetic variation. Numerous genes have been identified

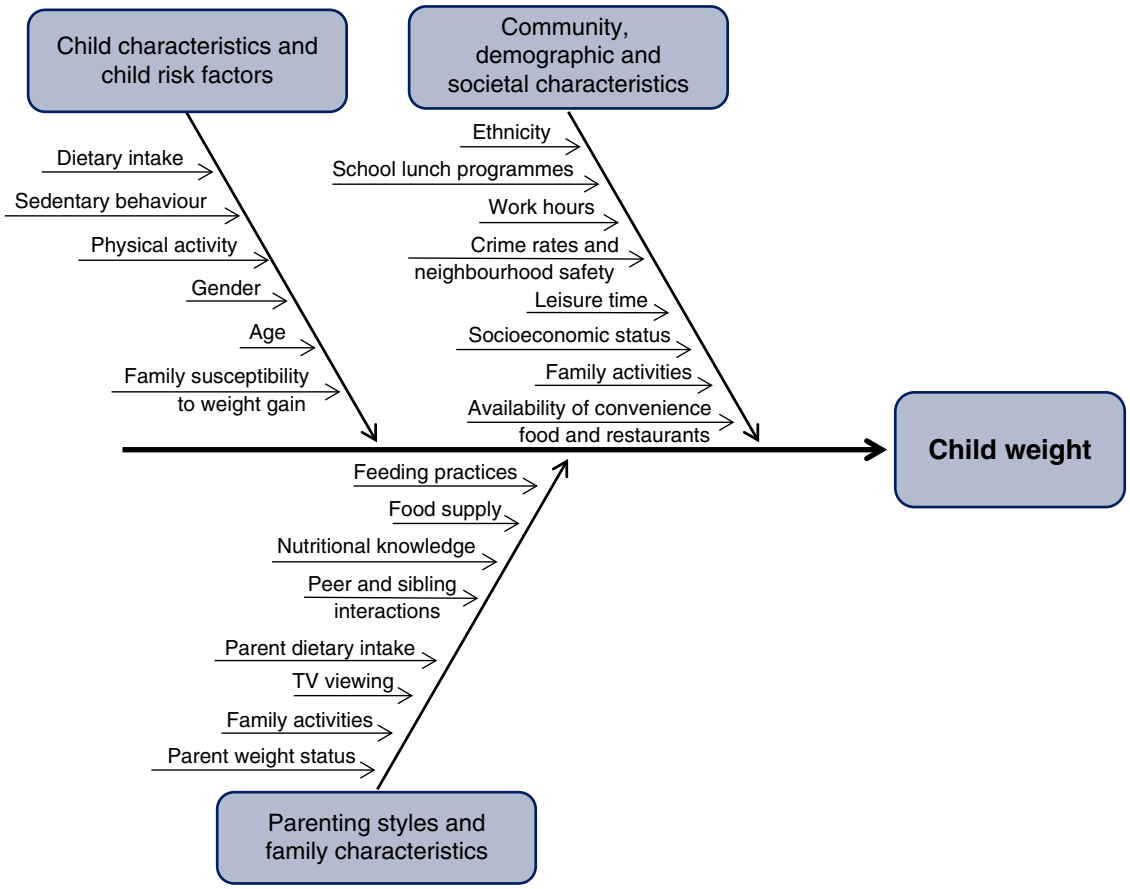

Figure 17.2 Causes of increasing overweight in children and adolescents. Child characteristics and risk factors, parenting styles and family characteristics as well as community, demographic and society characteristics are associated with the development of overweight children and adolescents.

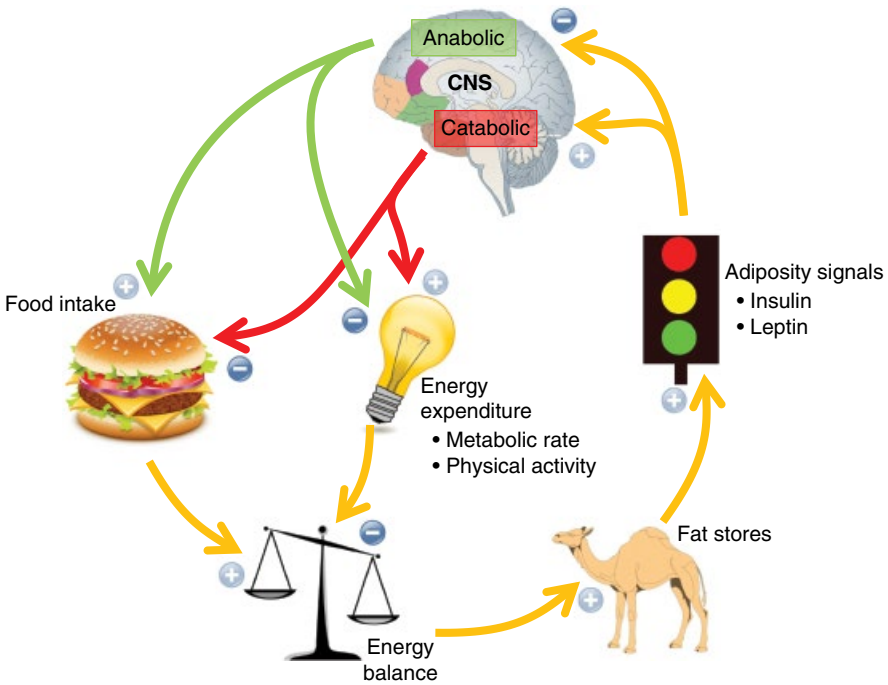

Figure 17.3 Interaction of food intake and energy homeostasis. There is a close interaction between the central nervous system (CNS), food intake, fat storage, adiposity signals, energy expenditure and balance to regulate energy homeostasis. (*See insert for colour representation of the figure.*)

Table 17.3 Genetic syndromes that may be associated with childhood obesity.

Albright's hereditary osteodytrophy (pseudohypoparathyroidism type 1a)
Prader-Labhard–Willi syndrome
MOMO (macrocephaly, ocular, mental retardation, obesity) syndrome
Alstrom syndrome
Bardet–Biedl syndrome
Carpenter syndrome
Börjeson–Forssman–Lehmann syndrome
WAGR (Wilms tumour, aniridia, genitourinary anomaly, mental retardation) syndrome
Cohen syndrome
ROHHAD syndrome (rapid onset obesity, hypothalamic dysfunction, hypoventilation, and autonomic dysfunction)
Primary (simple/'exogenous') obesity (multifactorial, multigenetic susceptibility)

by GWAS and candidate gene approaches that appear to be associated with the regulation of body weight. According to the thrifty gene hypothesis, evolutionary selection has selected genes that allow individuals to survive periods of food deprivation but, in an obesogenic environment, these traits have become detrimental.

The discovery of leptin and its receptors has stimulated research on obesity to a great extent. It is clear that this adipocyte product feeds back body fatness to the hypothalamus and regulates food intake in rodents. The fact that leptin serum concentrations are high in obese humans has led to the hypothesis that leptin insensitivity contributes to the further progressive development of obesity in overweight humans. On the other hand, leptin administration to children with leptin deficiency leads to a striking decrease of food intake, suppression of appetite, continuous weight loss and restoration of endocrine disturbances.

Mutations and polymorphisms in genes encoding other neuropeptides and hormonal regulators of appetite and weight control have been found in obese humans: mutations in the gene encoding *POMC* and polymorphisms of adrenergic receptors and melanocortin receptor (*MC4R*) have been shown to be associated with severe, morbid obesity. These genetic defects are the first monogenic disorders of weight control to be described and additional monogenic forms of obesity will no doubt be found; however a multifactorial aetiology of obesity will be present in most patients.

Common or Multifactorial Obesity and Fetal Programming

Less than 5% of cases of childhood obesity can be attributed to a specific cause and exogenous factors such as overconsumption of fat-rich diets, the excessive use of modern media (especially television) and a sedentary lifestyle (Table 17.1, Figures 17.2 & 17.3) all contribute heavily to the development of obesity in childhood and adolescence as well as in adulthood. Nutrition and diet early in infancy is thought to influence growth rate and body fatness beyond infancy. Some authors have suggested that intrauterine growth retardation predisposes for the development of obesity and syndrome X later in life. In fact both the intrauterine environment and growth during early life can influence the development of obesity and type 2 diabetes and hypertension, so-called fetal programming ('Barker hypothesis').

Intergenerational metabolic programming has also been discovered: for example, paternal diet determines weight development in the offspring, as shown in a *Drosophila* model where the sugar-rich diet of fathers before conception leads to obesity in offspring, an example of paternal-diet-induced/intergenerational programming. Although it is generally accepted that susceptibility to obesity is determined largely by genetic factors, the environment determines individual phenotypic expression.

Neurobiology of Satiety and Hunger

Neurons in the arcuate nucleus of the hypothalamus coordinate behavioural and autonomic functions that control energy homeostasis. Many genes that contribute to the aetiology of either monogenic or polygenic obesity (e.g. the genes encoding for the melanocortin 4 receptor [*MC4R*], *POMC*, CART [cocaine- and amphetamine-related transcript], neuropeptide Y [*NPY*], agouti-related protein [*ArRP*], the leptin receptor [*LEPR*], single-minded 1 [*SIM1*], brain-derived neurotrophic factor [*BDNF*], *TrkB* or *FTO*) are expressed in the hypothalamus (Figure 17.4). They are involved in the central regulation of energy intake, satiety, hunger and behavioural traits related to the control of food intake.

NPY/AgRP neurons provide orexigenic and antithermogenic signals while POMC/CART neurons provide anorexigenic and prothermogenic signals. These neurons also sense body energy stores and connect effector neurons to modulate caloric intake and energy expenditure. Variants in genes involved in energy utilization (e.g. those encoding β-adrenergic receptors 2 and 3, hormone-sensitive lipase and mitochondrial uncoupling proteins 1, 2 and 3) also contribute to common obesity through hypothalamic function. Other neuroendocrine modulators of energy balance include norepinephrine, serotonin and orexins A and B.

Under normal circumstances, the neuronal network of the hypothalamus ensures body mass stability over time and prevents both starvation and excessive body weight gain. Hypothalamic dysfunction has emerged as an important mechanism involved in the development of

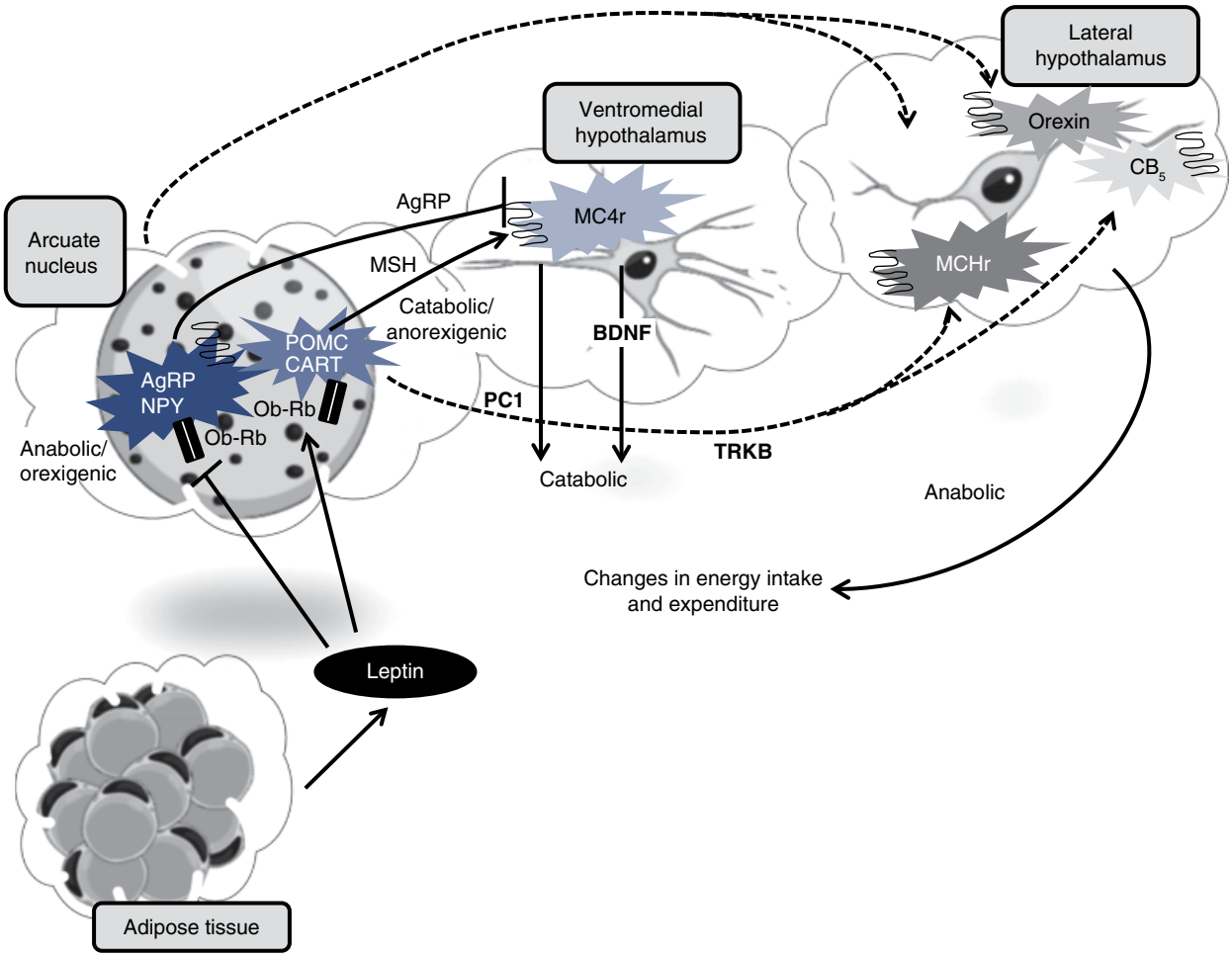

Figure 17.4 Human mutations involved in weight regulation. Leptin regulates feeding behaviour in the brain. Mutations of these signalling pathways alter feeding behaviour. AGRP, agouti-related protein; BDNF, brain-derived neurotrophic factor; CART, cocaine–amphetamine-regulated transcript; CB_5, cytochrome b5; MCHr, melanin-concentrating hormone receptor; MC4r, melanocortin 4 receptor; NPY, neuropeptide Y; POMC, pro-opiomelanocortin; TRKB, tyrosine receptor kinase B.

obesity and its co-morbidities, as exemplified by the obesity that develops after trauma to the hypothalamus (e.g. after surgery for tumours such as craniopharyngiomas). In addition, in one rare condition, the ROHHAD syndrome, **r**apid onset **o**besity is associated with **h**ypothalamic and **a**utonomic **d**ysfunction. Although satiety, food-seeking behaviour, obesity and caloric intake are generally linked to hypothalamic signalling, hypothalamic obesity has to be seen as a special and unique entity usually caused by direct damage to the hypothalamus. It is very difficult to treat.

Hedonistic Signals and Addiction

Selection of palatable meals, food intake and satiety causes pleasure and positive emotional, hedonistic signals. It is known that tetrahydrocannibinol stimulates food intake. The endogenous opioid system in the brain is linked to positive and motivating emotional signals. The central opioid system has long been known to regulate food intake and energy balance by modulating consumption beyond satiety; it is also involved in affective and stress responses and therefore positioned as a mediator between food intake, hedonistic responses and their emotional regulation. It has been hypothesized that obesity, overeating and food-seeking behaviour are features of addiction and dependency. The mechanism by which the central opioid system may regulate emotion and food intake and responses to acute food intake in humans is unknown. How the response of the opioid system is impaired in states of chronic overfeeding or obesity is also largely unknown. The question as to whether the opioid system may play a role in mediating the long-term maintenance of weight loss or counteract it has not been elucidated.

Positron emission tomography studies have shown that chronically obese humans have decreased receptor-mediated opioid binding potential and reduced activation of this neurotransmitter system in response to a standard meal than lean subjects, which suggests that the central opioid system relates not only to affective states and stress but also to chronic obesity and weight loss. Insulin and leptin alter energy balance and also modify the hedonistic pathway, namely, the pleasurable and motivating responses to food, which is the same central nervous system pathway that responds to drugs of abuse, such as morphine and nicotine. Food intake is considered a readout of the hedonistic, pleasurable pathway: when functional, the pathway will help reduce food intake and, when dysfunctional, will not do so.

Behaviour, Hypothalamus and Obesogenic Environment

The behavioural diversity of children includes a large variety of responses to the obesogenic environment because eating behaviour and responses to satiety, energy compensation, eating rate and meal frequency, responsiveness to food and food advertisements, food reward and taste and dietary preferences vary greatly among individuals. Hence, addressing behavioural aspects of childhood obesity should be considered an area of research and a target for intervention strategies but it has become apparent that no specific behaviour therapy is uniformly successful when applied to obesity and intended to result in weight loss and weight maintenance. It has been suggested that not only behavioural disturbance but also psychiatric disease such as attention-deficit disorder and depression as well as binge eating may lead to obesity even at a young age. Interaction of behavioural traits, such as reduced tolerance towards frustration and obesogenic environments, might lead to increased food intake and obesity.

Biology of Adipose Tissue and Adipocytes

Adipose tissue develops subcutaneously, viscerally and in organs. Fetal adipose tissue deposition is regulated by a complex interaction of transcription factors, many nutrients, and signalling molecules called adipocytokines. Maternal, endocrine and paracrine factors also influence specific changes in angiogenesis, adipogenesis and metabolism. During embryogenesis and in fetal life, leptin and adiponectin, two important adipocytokines, are present at high concentrations in the circulation and in tissues. In obese subjects, adiponectin serum concentrations are low while leptin serum concentrations are raised. Developmental stages and metabolic processes influenced by specific hormones and paracrine factors have been identified through examination of the offspring of obese and diabetic pregnancies, hormonal manipulation during late pregnancy in animal models and the use of cell cultures. Collectively, the results of these studies delineate the basis for imprinting or conditioning of fetal preadipocytes at the paracrine/autocrine level and of fetal adipose tissue development and metabolism.

Adipocytokines are hormones produced by and secreted from adipose tissue. Leptin was initially suggested as a promising 'anti-obesity' hormone but, in humans, leptin and its soluble receptor may be more important in states of energy deficiency rather than a predictor of the metabolic syndrome (MetS). Adiponectin, on the other hand, is not only related to obesity and insulin resistance but appears to be the strongest predictor for MetS, even in children. In newborns and infants, both adipocytokines occur in high concentrations, even though this cannot completely explain the increased risk for ensuing metabolic disease later in life (Figure 17.5).

Low-grade systemic inflammation and invasion by macrophages in adipose tissue may underlie the clustering of metabolic risk factors but their role in children remains to be elucidated. Overall, factors from the adipose tissue may constitute not only markers but also mediators of metabolic and cardiovascular consequences of obesity. Recent studies suggest the persistence of brown adipocytes in children, adolescents and even adult humans, as opposed to being exclusively present in

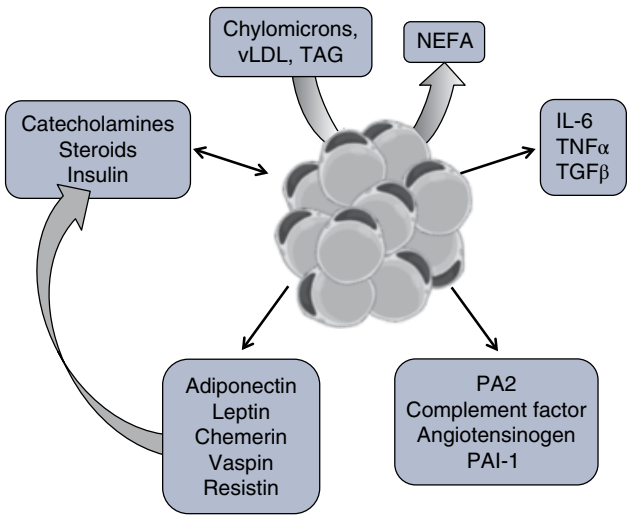

Figure 17.5 Regulators of adipose tissue. Extrinsic and intrinsic substances regulate adipose tissue function. IL-6, interleukin 6; NEFA, non-esterified fatty acids; PAI-1, plasminogen activator inhibitor-1; TAG, triacylglyceride; TGFβ, transforming growth factor; TNFα, tumour necrosis factor alpha; vLDL, very low-density lipoprotein.

infancy. Brown-like and UCP1, a marker of brown fat, positive adipocytes are interspersed within white adipose tissue perirenally, viscerally and subcutaneously in ~10% of lean children aged 0.3–10.7 years and detected by histological, immunohistochemical and expression analyses. No brown adipocytes are found in obese children or adults. Samples with brown-like adipocytes show an increased expression of a number of genes such as UCP1, PRDM16, PGC1α and CIDEA, all of which are involved in regulating cellular energy metabolism.

Many signalling molecules and metabolites are released from brown adipose tissue (BAT), and some are induced during brown adipocyte differentiation and/or thermogenic activation; some may have an autocrine or paracrine role. For example, nerve growth factor (NGF) and fibroblast growth factor 2 (FGF2) may increase sympathetic innervation and the number of preadipocytes in BAT. BAT also secretes non-peptide signalling molecules such as prostaglandins that may play a role in thyroid hormone function, differentiation of preadipocytes into beige adipocytes and synthesis of nitric oxide. Lack of BAT in obese individuals might then reduce energy expenditure and enhance energy storage in white adipose tissue.

Microbiota

Transfer of faecal microbiota from lean to obese individuals has been shown in one limited study to induce weight loss. Intestinal microbiota from obese rodents and humans differ from those from lean individuals. Acquisition of the microbiota in the gut begins at birth and a stable microbial community develops from a succession of key organisms. Disruption of the microbiota during maturation by low-dose antibiotic exposure can alter host metabolism and adiposity. For example, low-dose penicillin (LDP) delivered from birth induces metabolic alterations and affects ileal expression of genes involved in immunity. LDP that is limited to early life transiently perturbs the microbiota, which is sufficient to induce sustained effects on body composition, indicating that microbiota interactions in infancy may be critical determinants of long-term host metabolic effects. In addition, LDP enhances the effect of high-fat diet-induced obesity. Thus, early-life microbe-host metabolic interaction is consistently linked with metabolic alterations later in life.

Microbial colonization of mucosal tissues during infancy plays an instrumental role in the development and education of the host mammalian immune system. These early-life events have long-standing consequences by facilitating tolerance to environmental exposures or contributing to the development of disease in later life, including inflammatory bowel disease, allergy and asthma. Recent studies have begun to define a critical period during early development during which disruption of optimal host-commensal interactions can lead to persistent and in some cases irreversible defects in the development and training of specific immune subsets. There may very well be a 'window of opportunity', when microbial colonization has a potentially critical impact on human health and disease.

Faecal microbiome variation in the average healthy population has remained under-investigated. In one study, 14-genera core microbiota identified 664 genera and still underestimated total gut diversity. Sixty-nine clinical and questionnaire-based covariates were found associated with microbiota compositional variation, with a 92% replication rate. Stool consistency showed the largest effect size, whereas medication explained largest total variance and interacted with other covariate-microbiota associations. Early-life events such as birth mode were not reflected in adult microbiota composition. Finally, it was found that disease markers are associated with host covariates, underlining the importance of microbiota for maintaining health.

Insulin resistance is associated with ischaemic cardiovascular disease and type 2 diabetes. How the human gut microbiome impacts the serum metabolome and associates with insulin resistance has now been elucidated: the serum metabolome of insulin-resistant individuals is characterized by increased concentrations of branched-chain amino acids (BCAAs), which correlate with a gut microbiome that has an enriched biosynthetic potential for BCAAs and is deprived of genes encoding bacterial inward transporters for these amino acids. Some bacterial species such as *Prevotella copri* may be capable of inducing insulin resistance, aggravating glucose intolerance and augmenting circulating concentrations of BCAAs. It is suggested that microbial targets may have the potential to diminish insulin resistance and reduce the incidence of common metabolic and cardiovascular disorders.

Non-caloric artificial sweeteners (NAS) are among the most widely used food additives worldwide, regularly consumed by lean and obese individuals alike. NAS consumption is considered safe and beneficial owing to its low calorie content but supporting data remain sparse and controversial, although it has been shown that consumption of commonly used NAS formulations drives the development of glucose intolerance through the induction of compositional and functional alterations of the intestinal microbiota. These NAS-mediated deleterious metabolic effects are abolished by antibiotic treatment and are fully transferrable to germ-free mice by faecal transplantation of microbiota configurations from NAS-consuming mice or of microbiota anaerobically incubated in the presence of NAS. NAS-altered microbial metabolic pathways have been linked to host susceptibility to metabolic disease. NAS-induced

dysbiosis has been linked to glucose intolerance in healthy human subjects.

Endocrinology of Obesity

In order to understand the pathogenesis of co-morbidities and to find out what surrogate markers could be used to differentiate metabolically and cardiovascularly healthy from unhealthy obese individuals, many surrogate markers, adipocytokines, lipoproteins, lipids or growth factors and a variety of hormones, have been studied and related to obesity, its progression or its co-morbidities. For example, osteocalcin serum concentrations are inversely related to markers of body adiposity and leptin concentrations, and a relationship between obesity and bone health has been suggested. Associations between insulin-like growth factor 1 and IGF-binding proteins, bone turnover and obesity have also been studied.

The adipocyte appears to be a major site of extra-ovarian oestrogen synthesis. A longitudinal study noted that girls with greater BMI had lower serum oestradiol concentrations at the onset of breast development than those with BMI in the normal range. Previous studies have noted suppressed values of gonadotropins in obese girls. These findings suggest peripheral conversion of androgens into oestrogens through increased adiposity and greater aromatase activity. The association of obesity with increased bioavailability of hormones has also been noted. Studies have demonstrated a positive relationship of obesity to decreased sex hormone-binding globulin (SHBG), as well as increased androgen production. Decreased SHBG concentrations would lead to increased bioavailability of sex steroids and, coupled with increased aromatase activity, increased adrenal androgens could lead to increased peripheral conversion to oestrone, independently of the hypothalamic–pituitary–ovarian axis.

In most individuals, weight gain is the result of an 'obesogenic' environment superimposed on a background of genetic susceptibility brought about through evolutionary adaptation: such children with lifestyle-related obesity tend to be tall throughout childhood with advanced skeletal age, a process thought to be related to nutritionally driven increases in insulin-like growth factor 1 (IGF1). These children generally achieve their normal predicted adult height that may be related to an earlier onset and/or advanced progression through puberty.

Epigenetics

Obesity appears to change genetic expression in ways that favour the development of diabetes and cardiovascular disease and these changes may be passed to the next generation. Genes control an individual's phenotype but external forces can switch genes on and off, the epigenetic mechanisms that control how genes work. Epigenetic modifications may be involved in both obesity and development of T2DM. Epigenetics plays a pivotal role in the regulation of gene expression by the reversible modifications of chromatin structure without changes in DNA sequence. Epigenetic modifications include DNA methylation, post-translational histone modifications and miRNA interference. Many of the epigenetic changes associated with obesity-affected genes are known to raise diabetes risk.

In samples taken from obese subjects before and after gastric bypass, it was found that obese-type methylation patterns reverted to lean-type patterns. Exercise changes the epigenetic pattern of genes that affect fat storage and preliminary evidence suggests that epigenetic mechanisms in sperm could program a child towards increased weight gain and obesity that could explain part of the transgenerational, non-DNA-mediated transmission of obesity.

Social Inheritance

The sociocultural inheritance of body fat mass, weight and weight control also very largely contributes to the obesity epidemic obvious today. A quantitative analysis of the nature and extent of the person-to-person spread of obesity as a possible factor contributing to the obesity epidemic was, for example, carried out within a densely interconnected social network of 12,067 people repeatedly reassessed as part of the Framingham Heart Study (1971–2003); in this study, weight gain in one person was associated with weight gain in his or her friends, siblings, spouse and neighbours. A person's chances of becoming obese increased by 57% if he or she had a friend who became obese in a given interval and by 40% among pairs of adult siblings if one sibling became obese. Network phenomena therefore appear to be relevant to the biologic and behavioural traits of obesity, and obesity appears to spread through social ties. Thus, both intrinsic genetic and extrinsic socioeconomic and cultural factors contribute substantially and equally to the evolution of human obesity. As most children and teenagers grow up in families, parents might be well placed to facilitate and support weight management and prevent or manage obesity before adulthood.

Parental perceptions and views about their children's weight and the factors that influence their weight management strategies may influence a young person's weight development. The concerns of parents about their own weights provide a useful context for understanding their attitudes or actions with respect

to their children. Frustration about advising children and teenagers about weight management is frequently expressed and some parents worry about giving their growing child a 'problem' if they directly raise concerns about weight. Parent/teenage partnerships and parents creating a healthy home in which their offspring can make healthier choices are suggestions for interventions. The importance of taking parents' perceptions into account when developing family-based interventions to address teenage overweight and obesity is obvious.

Sociodemographics

In industrialized countries, the prevalence of obesity in children strongly relates to low income and low parental education but, in low-income countries (e.g. Brazil, India, China and the countries of the Arab peninsula), the reverse is seen and the children from the higher income part of the population are more frequently overweight and obese than children from a poor background. Whether urban environments pose a single risk over rural environments for children to become obese is debated: changes in the prevalence of obesity in Chinese children and adolescents in Shanghai, one of the most urbanized areas in China, were studied in a cross-sectional manner in 2003–2008. The standardized prevalence of overweight increased from 12.75 to 14.2% ($P < 0.01$) during the study period and the prevalence of obesity increased from 3.35 to 3.94% ($P < 0.01$) but, in contrast to data from developed countries, the prevalence of obesity decreased with age in both boys and girls. The prevalence of overweight and obesity in the urban area was significantly higher than that in the suburban area ($P < 0.01$). The high percentage of overweight and obesity in the younger age groups was of particular concern and urbanization might well be a causative factor for the increase in obesity prevalence in Chinese children in the Shanghai area.

Nutrition and Malnutrition

Dietary factors that may facilitate the development of overweight and obesity at a young age include total calorie, fat, protein and fibre intakes. In recent reviews, there was no consistent association of total calorie intake with overweight and obesity and a high calorie diet fed to rhesus monkeys led to higher concentrations of leptin and IGF-1, suggesting that there may be mechanisms that could lead to subtle influences on weight gain and hormonal homeostasis. Total dietary fat consumption may lead to more rapid weight gain, although there are inconsistencies in the data, and the type of fat (animal, polyunsaturated, etc.) may be important. Dietary protein was associated with earlier onset of weight increase and of the pubertal growth spurt, age of peak height velocity and menarche or voice breaking, and may be related to enhanced IGF secretion.

There has been a great deal of interest in the relationship of dietary fibre, weight and fat accumulation to the onset of puberty. Dietary fibre has also been recognized for its impact on cardiovascular disease, insulin resistance and diabetes and several cancers. In respect to the endocrine system, dietary fibre was noted to delay age of menarche and breast development, although other studies that documented lower overall intake of fibre did not note earlier menarche. Dietary fibre can lower concentrations of oestrogen through several mechanisms, which include increased faecal excretion through alteration in enterohepatic circulation of oestrogen, reduced bioavailability of oestrogen and suppressed gonadotrophin production. Given that traditional diets high in fibre would go along with calorically less dense foods or higher protein intake, some researchers have suggested that the relationship between greater fibre intake and lower bioavailable oestrogen might represent an evolutionary adaptation to delay reproduction when diet was suboptimal. On the other hand, low fibre might increase fat accumulation and earlier onset of reproductive potential. Because plant-based dietary intake is associated with phytoestrogen and fibre intake, studies are often challenged to separate the effects of phytoestrogens from other components of food, such as fibre.

The lack of association of calorie intake and obesity in many cohorts and epidemiological studies may be related to the inability of many assessment instruments to accurately measure food intake and food preferences but, while total caloric intake is not related to the development of overweight in children, snacking is. This highlights the fact that food intake behaviour is complex and a single measure such as calorie intake may not reflect the complex picture of overfeeding.

In many societies, overnutrition is related to malnutrition when lack of vitamins and trace elements as well as inadequate fibre adds to the disequilibrium of nutrition that will eventually lead to increased weight gain. Not only in developing countries but also in the USA, obesity and starvation are present in the same population and reflect the social inequalities present in these societies.

The automation of the process of extracting sugars in the 1900s reduced cost and increased availability of sugar, leading to a dramatic rise in consumption, which reached a peak in the 1970s. There are different definitions for sugars not naturally available in foods, and free sugars is the term used by the WHO. The epidemiological

evidence of the associations between sugars and obesity and type 2 DM is fairly strong and consistent, particularly for sugar-sweetened drinks in adults, but the sugar beverage industries as well as sugar industries have been quite successful in leading the public to believe that sugar-sweetened beverages are not strongly related to the obesity epidemic seen in parallel with the increasing consumption of sugar.

In the USA, fructose mainly from corn starch is added to sweeten many food stuffs. It is believed that high fructose intake might accelerate weight gain and also directly cause non-alcoholic liver disease and hepatic fibrosis. The Department of Health in the UK and many other countries have updated their recommendations for free sugars as a result of this scientific evidence and, in the UK, the recommended amount of free sugars is currently <5% of energy (reduced from <10%), which is difficult to meet and very different from current British dietary patterns.

Reducing free sugar intake is a challenge that will necessitate a range of different actions and policies. Public Health England has put forward eight suggestions, but the four most likely to improve dietary behaviour based on available evidence are social marketing, reduction of marketing of high sugar foods and drinks to children, reformulation and reductions in portion size and a sugar excise tax. Any action taken needs to be evaluated to check that inequalities are not widened. The new childhood obesity strategy has incorporated some but not all of these strategies and may not go far enough. It is unlikely that government policies alone will be sufficient and a change in the food culture is necessary to see real progress. It is unfortunate that not many countries have followed the UK example.

Physical Activity

Physical activity plays an important role in the prevention of overweight and obesity in childhood and adolescence and reducing the risk of obesity in adulthood. Puberty and the following adolescent period are particularly vulnerable times for the development of obesity due to a reduction in physical activity in many individuals. In many Western settings, a large proportion of children and adolescents do not meet recommended physical activity guidelines, and those who are more physically active have lower concentrations of body fat than those who are less active. Owing to the high risk of overweight adolescents becoming obese adults, the engagement of children and adolescents early in life in physical activity and sport is a fundamental goal of obesity prevention.

Neighbourhood and parental influences on diet and physical activity in young persons from low-income families have been studied and show that both are important mediators of adding to the obesity epidemic in many countries. For example, the relationship between neighbourhood characteristics and caregiver preferences for establishing diet and physical activity behaviours among low-income African American and Hispanic young children (2–5 years) has been explored in several studies. Neighbourhood constraints on desired behaviours, caregivers' strategies in response to neighbourhoods and caregivers' sense of agency in the face of neighbourhood constraints were crucial to the children's exercise and physical activity.

To address obesity disparities among young children, primary care behavioural interventions must encourage and support caregiver responses to neighbourhood constraints in order to address racial, ethnic and socioeconomic disparities in obesity among young children. Daily physical activity and obesity are inversely related in some studies but not in all, and low physical activity during weekends may relate to obesity and low income and lower education in families. Low-income families are more likely not to support sports activity and sports club membership of their offspring than are middle- or high-income parents.

Sedentary Behaviour

Sedentary behaviour is associated with health indicators but there are few studies in children that have examined these associations, especially in conjunction with psychological factors. One study from Japan examined the independent relationship between objectively assessed sedentary behaviour and indicators of obesity and psychological well-being, among elementary school children. 967 children completed a cross-sectional survey and sedentary behaviour was measured with accelerometers for seven consecutive days. Psychological well-being data in respect to anxiety and behaviour problems were collected. The amount of moderate-to-vigorous physical activity per day, duration spent wearing the accelerometer and degree of obesity were assessed: sedentary behaviour was significantly related to behavioural/emotional problems and there was a statistically significant relationship between sedentary behaviour and anxiety. By contrast, no significant association with degree of obesity was found.

Endocrine-Disrupting Chemicals and Toxicology

The involvement of environmental factors, such as endocrine-disrupting chemicals (EDCs), in the timing of onset of puberty has received much interest. Evidence for this comes from recent changes in age at puberty

onset and pattern of distribution that vary among countries, as well as forms of sexual precocity after migration; however the evidence of association between early or late pubertal timing and exposure to EDCs is weak in humans. Feedback adds to the complexity of regulation so that changes in pubertal timing caused by EDCs could involve both central and peripheral mechanisms.

EDCs may cause disturbed energy balance and account for the obesity epidemic, which adds another layer of complexity to understanding the role that they may play in the timing of puberty. Several aspects link this system and the reproductive axis that leads to coexisting neuroendocrine and peripheral effects and has a dependency on fetal/neonatal programming. Many factors have been discovered that cross link the two systems including leptin, adiponectin and agouti-related peptide (AgRP).

EDCs are frequently seen as obesogens; they represent several classes of chemicals that may impact on early weight gain, metabolic consequences later in life and timing of puberty through regulating peroxisome proliferator-activated receptor gamma (PPARγ) or through modulation of aromatase. These chemicals have been proposed to promote adipogenesis (adipocyte number and/or size) through modulating signalling pathways or through stimulation of mesenchymal stem cells to preadipocytes and maturation of preadipocytes into adipocytes. The fungicide triflumizole, for example, was noted in animal models to upregulate PPARγ and induce mesenchymal stem cells to express adipocyte markers, and prenatal exposure to tributyltin led to increased adipose tissue mass, increased adipocyte size and number and increased expression of adipocyte cell markers in mesenchymal stem cells.

Media Use

Today's children and adolescents are immersed in both traditional and new forms of digital media. Research on traditional media, such as television, has identified health concerns and negative outcomes that correlate with the duration and content of viewing. Television viewing, daily duration of viewing and particularly viewing during evenings after evening meals are all associated with higher weights and the development of obesity, even in young children. The use of digital media, including interactive and social media, has grown and research evidence suggests that these confer both benefits and risks on the health of children and teenagers. Evidence-based benefits include early learning, exposure to new ideas and knowledge, increased opportunities for social contact and support and new opportunities to access health promotion messages and information. Risks include negative health effects on sleep, attention and learning; a higher incidence of obesity and depression; exposure to inaccurate, inappropriate or unsafe content and contacts and compromised privacy and confidentiality.

Cultural Aspects

In some countries and societies, being well fed, overweight and even being obese indicates wealth and prosperity and may suggest fecundity and longevity. Cultural and health beliefs are strong indicators of a society's prosperity and longevity. Many dietary interventions assume a positive influence of home cooking on diet, health and social outcomes but evidence remains inconsistent.

Evolution

Evolutionary medicine may induce a conceptual revolution in medicine but the field does not have the same theoretical rigour nor the direct and obvious clinical relevance as in many other fields in medicine. Starting with the thrifty gene hypothesis, evolutionary thinking in obesity and type 2 diabetes has undergone several transitions, modifications and refinements. According to the hypothesis, genes that helped to survive starvation in ancient times will lead to fat accumulation and obesity in today's world of food abundance.

Alternative hypotheses independent of thrift have also been suggested but most look at partial pictures and make selective use of supportive data while ignoring inconvenient truths. Most look at a superficial picture and avoid getting into the intricacies of underlying molecular, neuronal and physiological processes. Very few have suggested clinical implications and none has been tested with randomized clinical trials.

Explanations of why obesity is more prevalent in some sectors of the human population than others are lacking. Although it is well recognized that obesity is a result of gene–environment interactions and that predisposition to obesity lies predominantly in our evolutionary past (thrifty genes), there is much debate as to the precise nature of how our evolutionary past contributed to obesity. The 'drifty genotype' hypothesis suggests that the prevalence of thrifty genes is not a result of positive selection for energy-storage genes but attributable to genetic drift resulting from the removal of predative selection pressures.

Both theories assume that the pressures the ancestors of modern humans living in Western societies faced were the same. Neither theory adequately explains the impact of modern globalization and changing population demographics on the genetic basis for obesity in developed

countries, despite clear evidence for ethnic variation in obesity susceptibility and related metabolic disorders. It is hypothesized that the modern obesity pandemic is a result of the differential exposure of the ancestors of modern humans to environmental factors that began when modern humans left Africa around 70,000 years ago and migrated through the globe, reaching the USA around 20,000 years ago. How an understanding of ethnic differences in genetic susceptibility to obesity and the MetS could be used in the treatment of obesity in industrialized countries needs to be clarified.

One alternative candidate explanation of the evolution of obesity is the insurance hypothesis that suggests that the function of storing fat is to provide a buffer against shortfall in the food supply. Applied to humans, this implies that an important proximate driver of obesity should be food insecurity rather than food abundance. A theoretical model that shows it is optimal to store more fat when food access is uncertain has been proposed. Evidence from animal studies show that fat reserves increase when access to food is restricted. There is a robust positive association in humans as well but it is restricted to adult women in high-income countries. While the insurance hypothesis alone cannot explain the distribution of obesity in the human population, it may represent an important component of a pluralistic explanation.

Epidemiology

Current and projected obesity rates (BMI $\geq 30\,kg/m^2$) in the adult population of the USA are 20% for the year 2000, 30% for 2015 and >40% for 2025. The Bogalusa Heart Study found that 22% of the children surveyed in 1990 had a BMI > 85th centile established in 1980. There was little change in the cohorts of children with a BMI < 50th centile so obese children had a tendency towards even more excessive weight over time and childhood overweight and obesity is commonly seen across most developed and developing countries with a prevalence of about 20–35%.

Developed Countries

The prevalence of obesity in young children is plateauing or even declining slightly in many industrialized countries such as Sweden, France, Germany and Australia, but obesity prevalence in adolescents is still increasing throughout the world. Data from 272,826 German children aged 4–16 years between 1999 and 2008 were analysed. A significant upwards trend for overweight and obesity prevalence was found between 1999 and 2003 and a significant downwards trend between 2004 and 2008. Prevalence increased in most subgroups until 2004 but a downwards trend was found in children aged 4–7.99 years, whereas it stabilized in most other subgroups. When data from 93,028 children were analysed in cross-sectional analyses, the prevalence was significantly higher in girls aged 12–16 years and in boys aged 8–16 years in 2004 compared with in 2000 but significantly lower in 2008 compared with in 2004 in children aged 4–7.99 years.

Developing Countries

The prevalence of overweight and obesity in children has been dramatically increasing in developing and poor countries. Obesity is found in parallel with malnutrition and undernutrition in the same population in different social strata but even within the same social class in some countries. These findings suggest a common influence of low education, lack of knowledge about healthy foods and the importance of physical activity that affects the physical development of children. Lack of prenatal care and low birth weight may add to an increased fat accumulation at an early age.

Co-Morbidities and Consequences

Among the common sequelae of primary childhood obesity are hypertension, dyslipidaemia and psychosocial problems. A more complete list of co-morbidity disorders is shown in Table 17.4. These disorders, which arise from overweight tendencies and subsequent biochemical changes, actually predispose to additional co-morbidity such as cardiovascular disease in early adulthood. 60–85% of obese children of school age stay obese in adulthood and the co-morbidities are a major health burden to industrialized societies. Childhood obesity seems to increase the risk of subsequent morbidity, whether obesity persists in adulthood or not. It is, therefore, mandatory to examine obese children in respect to the presence of co-morbidity even at a young age. Such examinations should include blood pressure monitoring and checking of lipid status. The opinion of orthopaedic surgeons and child psychiatrists should be sought much more frequently. Most importantly, type 2 diabetes, which until recently had been considered a disorder of the older population, is being increasingly recognized in children and adolescents, especially in children of African American and Hispanic ethnicity. The clinical picture in these children and the fact that most affected patients come from families with type 2 DM have led

Table 17.4 Co-morbidity of obesity in childhood and adolescence.

Psychosocial – psychiatric
Poor self-image
Social isolation
Autoaggression
Suicide
Promiscuity
Drug and alcohol addiction
Bulimia
Binge eating
Smoking
(Enuresis)
Cardiovascular and respiratory
Accelerated atherosclerosis
Hypertension
Hypoventilation
Sleep apnoea
Snoring
Obstructive lung disease
Obstructive sleep apnoea syndrome, Pickwickian syndrome
Reduced lung capacity
Endocrine, metabolic and gynaecological
Hyperinsulinaemia
Insulin resistance
Early puberty
Polycystic ovaries
Dysmenorrhea
Dyslipidaemia
Orthopaedic
Slipped capital femoral epiphyses
Coxa vara
Blount's disease
Legg–Calve–Perthes disease
Back pain
Dermatological
Paronychia
Acanthosis nigricans
Striae rubrae

physicians to conclude that affected children will respond to the same treatments used in adults and that clinical courses will be similar to those described in adults but there are few published data about experience in children with most of the drugs that are used for glycaemic management in adults with type 2 diabetes. In most countries, only metformin is approved for the treatment of type 2 diabetes in youth.

Carbohydrate Metabolism

The prevalence of the MetS in obese children is reported to be 30%, irrespective of the definition applied. Insulin insensitivity often leads to type 2 diabetes and represents one of the most clinically relevant co-morbidities of obesity that has long been the focus of obesity research. Hyperinsulinaemia indicating insulin resistance and glucose intolerance is found in ~30% of obese children and adolescents. There is a strong relation between cardiorespiratory fitness and markers of insulin insensitivity. Maintenance and/or improvement of cardiorespiratory fitness prevents the development of insulin insensitivity and hence the importance of physical activity on metabolic health.

A link between thyroid function, obesity and insulin insensitivity has also been reported. It appears that children with higher TSH concentrations have higher degrees of insulin insensitivity as expressed by HOMA-IR.

Type 2 diabetes in relation to obesity has become the most frequent form of diabetes in children with Asian, Hispanic and African American ethnic backgrounds, but type 2 diabetes in the obese usually develops only after 10 years of age and not before adolescence in white subjects. Puberty leads to increased insulin concentrations and may add to the development of insulin resistance. Type 2 diabetes can occur without any symptoms, and health problems associated with the disease are serious even early in life; however, screening tests allowing early diagnosis, although desirable, are controversial. In one observational multicentre analysis including 4848 (2668 female) overweight and obese children aged 7–17 years without previously known diabetes, HbA1c and OGTT were used as diagnostic criteria and 2.4% (n = 115; 55 female) were classified as having diabetes. Within this group, 68.7% had HbA1c concentrations ≥48 mmol/mol (≥6.5%). Fasting plasma glucose ≥126 mg/dL (≥7.0 mmol/L) and/or 2 hour glucose concentrations ≥200 mg/dL (≥11.1 mmol/L) were found in 46.1% of cases. Of the 115 cases fulfilling the OGTT and/or HbA1c criteria for diabetes, diabetes was confirmed in 43.5%.

For HbA1c, the best cut-off value was 42 mmol/mol (6.0%) with a sensitivity of 94% and a specificity of 93%, and HbA1c seems to be more reliable than OGTT for diabetes screening in overweight and obese children and adolescents.

The longitudinal course of impaired glucose metabolism has been assessed by oral glucose tolerance test (oGTT) in a large multicentre pediatric obesity registry. oGTT data were analysed in obese children in an observational multicentre (n = 84) cross-sectional (n = 11,156) and longitudinal analysis (n = 1,008). Patients were stratified with impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and type 2 diabetes (T2D), according to American Diabetes Association criteria. 12.6% of the children presented with abnormal glucose metabolism (5.99% IFG, 5.51% IGT, 1.07% T2D). BMI correlated modestly with 2 hour blood glucose (r = 0.04, P < 0.001). In the 1008 patients with follow-up oGTT,

metabolic parameters improved and the percentage of abnormal glucose metabolism decreased from 18.7 to 14.2%. Of the children with initial IGT, 70.6% reverted to normal glucose tolerance and the improvement was associated with, but not dependent on, a reduction of BMI SD score.

Lipid Metabolism

Overweight and obesity are correlated with an increased risk of cardiovascular and/or metabolic diseases. Cardiovascular diseases usually manifest after the fourth decade of life but atherosclerosis starts at an early age, for which abnormal serum lipids are crucial risk factors. In a population-based German cohort of children and adolescents (LIFE-Child cohort), the prevalence of dyslipidaemia between 0 and 16 years is 6–22%. In this study, no significant correlation between total or LDL cholesterol and social class or family wealth was detected, but BMI and body weight SDS were strongly related to serum lipid concentrations. A study from Brazil of young adults aged 23–25 years showed a significant ($P < 0.05$) influence of social status on total cholesterol, LDL and HDL cholesterol, but a study from Finland reported higher HDL concentrations in children and adolescents of higher social status implying that children of lower social status would have a higher cardiovascular risk; indeed they were found to have significantly higher triglyceride concentrations. This inverse relationship was also reported in other studies.

Apolipoprotein B is one of the most important predictors for the development of hypercholesterolaemia within a 5-year period and, with higher social status, concentrations of ApoB have been shown to decrease, which in turn is associated with a lower cardiovascular risk. The increased risk of CHD in obesity was mediated, among other factors, through increased concentrations of ApoB and decreased ApoA1. The significantly higher ApoA1 concentrations in subjects with higher socioeconomic status (SES) or increased family affluence underline the cardioprotective advantages in this social stratum, but poorer people with good education and access to good nutrition and physical exercise opportunities may have the same health prospects as wealthier people.

Cardiovascular

Obesity in youth is linked to higher blood pressure and is associated with cardiovascular risk, carotid intima-media thickness and myocardial structural alterations that may influence cardiac mechanics. In a study of 61 obese (13.5 ± 2.7 years of age, 46% male sex, SD score BMI, 2.52 ± 0.60) and 40 non-obese (14.1 ± 2.8 years of age, 50% male sex, SD score BMI, −0.33 ± 0.83) Caucasian children and adolescents, standardized 2-dimensional (2D) echocardiography and 2D speckle-tracking analysis was performed with blood lipid and glucose analysis. Blood pressure, low-density lipoprotein cholesterol and parameters of glucose metabolism were significantly increased in obese children, whereas high-density lipoprotein cholesterol was significantly lower. Compared with non-obese children, obese children had enlarged left- and right-side cardiac chambers, thicker left ventricular walls and, consequently, increased left ventricular mass. Despite a comparable left ventricular ejection fraction, decreased tissue Doppler-derived peak systolic velocity and regional basoseptal strain were found in obese children compared with non-obese children. 2D speckle-tracking-derived longitudinal and circumferential strain of the left ventricle was reduced in obese children and diastolic function was also impaired. Longitudinal and circumferential strains were both independently associated with obesity. In short, childhood obesity is associated with significant changes in myocardial geometry and function, indicating an early onset of potentially unfavourable alterations in the myocardium.

Skeletal System

There is a higher prevalence of orthopaedic conditions such as joint and back pain in overweight and obese children. As physical activity is directly related to improvements in physical fitness, skeletal health and metabolic conditions, higher levels of physical activity are encouraged and exercise commonly prescribed in the treatment and management of childhood obesity. Research has not correlated orthopaedic conditions with decreases in physical activity but overweight children typically display a slower, more tentative walking pattern with increased forces to the hip, knee and ankle during 'normal' gait that suggests that such individuals are poorly equipped to undertake certain forms of physical activity.

Recent evidence suggests an increased association between obesity and musculoskeletal pain and increased fracture risk due to decreased bone mineral content and impaired bone structure. The limitations imposed by increasing body mass appear to be directly reflected in the child's level of activity and overall functional capacity of the musculoskeletal system. The available data suggest that obesity affects the child's locomotor system both functionally and structurally.

Non-Alcoholic Fatty Liver Disease (NAFLD, NASH)

Despite the fact that NAFLD is closely linked to the MetS, it has so far rarely been discussed as an additional component of the syndrome. Less than 5% of the liver consists of lipids, and fatty infiltration in excess of this confirmed by liver histology and in the absence of excessive alcohol intake and viral, autoimmune and drug-induced liver disease is defined as NAFLD. NAFLD spans from simple hepatic steatosis to non-alcoholic steatohepatitis (NASH) with or without fibrosis and this can evolve to hepatic cirrhosis and its complications such as hepatocellular carcinoma and portal hypertension.

NAFLD due to obesity is the most common form of liver disease in children aged 2–19 years. Its prevalence has more than doubled in the past 20 years. The prevalence in children and adolescents varies from 3 to 11% and is strongly influenced by age, sex, race and ethnicity. In obese children, the prevalence ranges from 38 to 80%. NAFLD prevalence increases with age, a prevalence of 0.7% for ages 2–4 years rising to 17.3% for ages 15–19 years. Fatty liver disease appears twice as often in boys than in girls and, according to some authors, the prevalence is highest in American Hispanics (45%), intermediate in Caucasians (33%) and lowest in African American (24%). In Europe, Australia and the Middle East, the prevalence ranges from 20 to 30%, which is comparable with that reported from Japan, China, Latin America and India. It can be concluded that obese Hispanic boys are at the greatest risk of developing NAFLD at an early age.

The pathogenesis of NAFLD is thought to be a multiple-hit process involving insulin resistance, oxidative stress, apoptosis and adipocytokine action. Although the pathophysiology of NAFLD is not fully understood, a 'two-hit hypothesis' for the development of NAFLD and NASH has been put forward. First, hepatic triglyceride accumulation increases the susceptibility for liver injury and then 'the second hit' may involve inflammation, mitochondrial dysfunction and oxidative stress, which will eventually lead to steatohepatitis and fibrosis.

Metabolic Syndrome

In 2004 the International Diabetes Federation (IDF) published a definition of the MetS in adults that included hypertension, dyslipidaemia, glucose intolerance and abdominal obesity as constituents. This cluster of risk factors is related to the development of cardiovascular diseases and type 2 diabetes. MetS has also been described in children and adolescents but there is no consensus definition of the MetS in children. In particular, there is a lack of reference values for its different components during early life but definitions of the MetS at a young age have been put forward in recent years by a number of groups and learned societies.

Every single definition has its strengths and weaknesses but all agree that the MetS would apply if a child has abdominal obesity, high blood pressure, abnormal blood lipids and disturbed glucose/insulin metabolism. Heterogeneous parameters and cut-off limits of each of the constituents have been used for the respective definitions. There is a tendency to classify MetS in children with only three of four components being present.

Clearly the prevalence of MetS in children varies depending on the definition used. In the IDEFICS (**I**dentification and prevention of **D**ietary- and lifestyle-induced health **EF**fects **I**n Children and infant**S**) cohort, a large population-based sample of European children aged 2–9 years, the prevalence of MetS varied between 0.3 and 1.5% in girls and between 0.4 and 1.3% in boys. In order to avoid these discrepancies, a new definition based on the results from the IDEFICS cohort has been suggested using age- and sex-specific centiles for each of the components and two different cut-offs were agreed to help pediatricians decide whether a child needs close monitoring or intervention.

If the centile values of at least three of the four components of MetS exceed the 90th or fall below the 10th for HDL cholesterol, children need monitoring, but, if the values are >95th or <5th for HDL cholesterol, they need intervention. Parameters used by the IDEFICS investigators to establish a diagnosis of MetS included WC to characterize the adiposity level. WC seems to be the best predictor of visceral adipose tissue. WC also seems a reliable predictor of elevated systolic blood pressure (SBP) and insulin resistance. Insulin resistance is often defined using the HOMA index. Both systolic and diastolic blood pressures are measured and serum lipids including triglycerides (TG) and total cholesterol as well as LDL and HDL cholesterol concentrations are assessed. Blood glucose (fasting plasma glucose) and insulin determinations are also included in order to assess the presence or absence of MetS.

Recently, a scoring system for the presence and severity of MetS in early life has been suggested. The continuous score combines the components used to define MetS in adults by summarizing the age- and sex-standardized z scores of WC, systolic and diastolic blood pressure, TG, HDL cholesterol and HOMA data. The MetS score is calculated as $z\text{WC} + (z\text{SBP} + z\text{DBP})/2 + (z\text{TRG} - z\text{HDL})/2 + z\text{HOMA}$; the higher the score, the less favourable is the metabolic profile.

Urogenital

An increased prevalence of urogenital infections including bacterial infections of the kidney and non-specific cystitis, balanitis and vulvovaginitis has been suggested in obese subjects, and outcomes of renal disease are thought to be less favourable in obese and overweight children. Polycystic ovarian syndrome is characterized by ovarian cysts, obesity and hyperandrogenism. Pseudoprecocious puberty is thought to be misdiagnosed in obese girls: pelvic ultrasound evaluation of girls presenting with secondary sex characteristics before the age of 8 years may help to differentiate precocious puberty from premature thelarche, functioning ovarian cyst and obesity but, although uterine and ovarian measurements are significantly higher in girls with true precocious puberty than controls, there is a significant overlap of normal prepubertal and early pubertal values.

Puberty and Fertility

Many cross-sectional analyses and longitudinal studies have examined the association between body weight, height, BMI, fat mass and skinfold thickness and pubertal development. In addition, the possible impact of adiposity on reproduction and fertility in obese males and in male animal models of obesity has been studied. A trend towards earlier pubertal development and maturation in obese individuals of both sexes has been shown and the previously held notion that obese boys might progress to puberty at a slower pace than their non-obese peers can no longer be substantiated.

Impaired fertility markers and reduced reproductive function have been observed both in obese humans and in animal models of obesity; leptin-deficient mice have central hypogonadism and reduced fertility and low sperm counts and reduced sperm function have been reported in obese men. Adipocytokines and inflammatory cytokines have all been shown to impact on reproductive function and directly interact with the male reproductive system. For example, nicotinamide phosphoribosyltransferase (NAMPT), a key enzyme of NAD metabolism, is not only released from adipocytes but also expressed in the testis (Table 17.5). It is thought that NAMPT might be a key player in modulating sperm function in the human.

It has been hypothesized that the pandemic of childhood obesity may have caused earlier maturation in both sexes. Over the years, a positive trend in both height and weight has been observed in a study from Belgium of children aged over 5 years. Adult median heights have increased by 1.2 cm/decade and median weights by 0.9 kg/decade in boys and the weight distribution has become more skewed; however, the onset of pubertal stages of G2 (enlargement of scrotum and testes) was comparable with that reported from other Western European countries.

Table 17.5 Hypothetical mechanisms of how obesity might affect onset and tempo of pubertal development and sexual function.

- Common genes linking energy metabolism and puberty
- Nutritional factors (protein, sugars)
- Energy supply (calories)
- Adipocytokines (signalling)
- Insulin/insulin-like growth factor effects (i.e. on ovary)
- Nicotinamide phosphoribosyltransferase (NAMPT) and other enzymes providing increased amounts of energy to gonads

There is still much debate regarding the true impact of childhood obesity on testicular development in boys. A link between fertility and testicular function and obesity or fat mass has been suggested but not proven. Molecular mechanisms substantiating the link between fat mass and sperm function are not known. Endocrine disruptors may very well cause both increased weight gain and at the same time reduce sperm counts, motility and hence fertility in men. Adrenal androgens may represent a critical link between body fatness and timing of puberty but whether or not adrenarche impacts upon the timing of pubertal development is controversial.

The association of adrenal androgen secretion with early and late pubertal markers, independent of potential influences of dietary animal protein intake, was investigated in a prospective cohort study of healthy Caucasian children (n = 109), who provided 24 hours urine samples and 3 days of weighed dietary records, 1 and 2 years before the biological age at take-off of the pubertal growth spurt. Twenty-four-hour excretion rates of androgen (C19) metabolites were quantified. Taken together, higher animal protein intake may be involved in an earlier attainment of age at take-off of the pubertal growth spurt and age at peak height velocity, whereas a more intensive adrenarchal process may precipitate a shorter pubertal growth spurt and a notably earlier onset of breast and genital development in girls and boys, respectively.

Genetic variants may also account for a link between obesity and pubertal timing. Variations in LIN28B, a human ortholog of the gene regulating processing of micro-RNAs, were recently reported to be associated with timing of puberty in humans. Genetic variation in LIN28B was associated with earlier voice breaking and more advanced pubic hair development in boys. In addition, a faster tempo of height attainment in girls and boys and a shorter adult height in those with allelic variation at this site, in keeping with earlier growth cessation, was documented. These data suggest that genetic influences on pubertal timing may be important and stronger than

environmental factors, the impact of which may be more difficult to assess.

Many additional studies indicate that the timing of pubertal onset is under strong genetic control but genes controlling pubertal timing in the general population have not been identified. A genome-wide association scan detected linkage of constitutional delay to a region on chromosome 2p13–2q13 and the pericentromeric region of chromosome 2. This locus may therefore represent a component of the internal clock controlling the timing of onset of puberty.

Another important mechanism that contributes to the regulation of puberty by body composition is the interrelationship between the gut, fat and central nervous system hormones and peptides (Figure 17.6). There has been an increased understanding of how hormones and gut peptides affect energy intake and storage in adipose tissue and how these interact with the central nervous system to control energy balance, and some of these peptides play an important role in modulating the gonadotropic axis; their absence or an imbalance in their secretion may disturb pubertal onset or progression.

A growing body of evidence from rodent and human studies suggests that leptin may be the critical link between body fat and earlier puberty. Leptin-deficient mice and humans fail to enter puberty unless leptin is administered and rodent studies indicate that very low concentrations of leptin stimulate gonadotropin secretion both at the hypothalamic and the pituitary levels. Current evidence, however, indicates that leptin appears to play a permissive role rather than act as the critical metabolic signal initiating puberty. Knowledge concerning the role played by ghrelin, the only orexigenic and growth-promoting peptide produced by the digestive tract, on sexual development has also been accumulating and other peptides derived from the digestive tract may also be involved in the regulatory link between energy homeostasis and sexual development.

Psychology and Psychiatric Co-Morbidity

There are a number of common psychological conditions associated with childhood obesity. A systematic search on MEDLINE, Web of Science and the Cochrane Library shows that overweight and obesity at a young age is associated with depression, lower scores on health-related quality of life, emotional and behavioural disorders and low self-esteem during childhood. Evidence of an association between attention-deficit/hyperactivity disorder (ADHD) and obesity remains unconvincing because of contradictory findings from different studies.

Overweight children are more likely to experience psychosocial problems than their healthy-weight peers, which may be adversely influenced by obesity stigma, teasing and bullying, which are pervasive and can have serious consequences for emotional and physical health and performance. Whether psychiatric disorders and psychological problems are a cause or a consequence of childhood obesity or whether common factors promote both obesity and psychiatric disturbances in susceptible children and adolescents is unclear.

Social Participation

It has been hypothesized that obese children are less active in participating in social interaction with peers or in their community in general. Social network analysis has been used to examine whether peer influence from one's social networks moderates obesity prevention programme effects on obesity-related healthy and unhealthy behaviours, and results indicated that peer exposure was positively associated with one's own behaviours. Programme participation effects could be adversely influenced by unhelpful peers whose influence can probably diminish or (hopefully) amplify prevention programmes. For prevention, future programmes should consider peer-led components to promote a helpful influence of peers on behaviours, and programmes

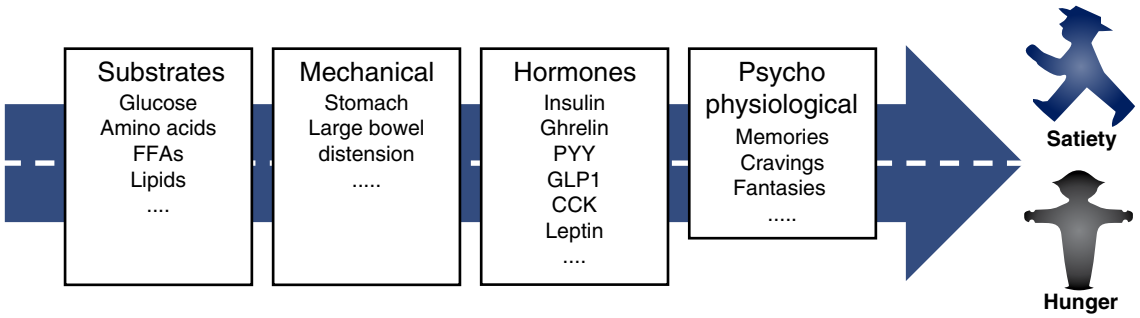

Figure 17.6 Physiological response to food. Substrates, hormones, mechanical and psychophysiological quantities influence satiety or hunger. CCK, cholecystokinin; FFA, free fatty acid; GLP1, glucagon-like peptide; PYY, peptide YY.

should be mindful of the fact that their effects are moderated by social networks.

Mortality

There are many interactions between major sociodemographic and behavioural risk factors associated with all-cause mortality. There are two forms of interaction between risk factors: additive and multiplicative relations. Usually, expectations about interactions among sociodemographic variables and their relation to behavioural variables have been stated in terms of additivity but the statistical models typically used to estimate the relation between risk factors and mortality assume that risk factors act multiplicatively. Therefore, the nature of interactions among the five major risk factors associated with all-cause mortality, smoking, obesity, race, sex and educational attainment, has to be seen as a complex multifaceted interaction. Obesity has been found to be additive with each of the other variables. It is speculated that its additivity is a reflection of the fact that obese status is generally achieved later in life.

For all pairings of sociodemographic variables, risks are multiplicative. For survival chances, it is much more dangerous to be poorly educated if one is black or male and much riskier to be a male if one is black. These traits, established at birth or during childhood, literally result in deadly combinations.

The association between childhood body weight and adult health has been little examined and findings are inconsistent. In a representative sample of the Scottish nation (the Scottish Mental Survey of 1947), the association between BMI measured at 11 years of age and future cause-specific mortality by age 77 years was studied. In this cohort study with a maximum of 67 years and follow-up of 3839 study members, there were 1568 deaths (758 from cardiovascular disease, 610 from any malignancy). After adjustment for covariates, there was some evidence of a relation between elevated childhood BMI and rates of mortality ascribed to all causes (hazard ratio (HR) per 1 SD increase in BMI, 95% confidence interval: 1.09; 1.03, 1.14), cardiovascular disease (1.09; 1.01, 1.17), all cancers combined (1.12; 1.03, 1.21), smoking-related cancers (1.13; 1.03, 1.25) and breast cancer in women (1.27; 1.04, 1.56).

The association of BMI in late adolescence with diabetes mortality in midlife has also been studied: the BMI values of 2,294,139 Israeli adolescents (age 17.4 ± 0.3 years), measured between 1967 and 2010, were grouped by US Centers for Disease Control and Prevention age/sex centiles and by ordinary BMI values. The outcome, obtained by linkage with official national records, was death attributed to DM as the underlying cause. During 42,297,007 person years of follow-up (median, 18.4 years; range <1–44 years), there were 481 deaths from DM (mean age at death, 50.6 ± 6.6 years). There was a graded increase in DM mortality evident from the 25th to the 49th BMI centile group onwards and from a BMI of 20.0–22.4 kg/m^2 onwards. Overweight (85th–94th centiles) and obesity (the 95th centile or higher), compared with the 5th–24th centiles, were associated with HRs of 8.0 (95% CI 5.7–11.3) and 17.2 (11.9–24.8) for DM mortality, respectively, after adjusting for sex, age, birth year, height and sociodemographic variables. The HR for the 50th–74th centiles was 1.6 (95% CI 1.1–2.3). Findings persisted in a series of sensitivity analyses. The estimated population-attributable fraction for DM mortality, 31.2% (95% CI 26.6–36.1%) for the 1967–1977 prevalence of overweight and obesity at age 17, rose to a projected 52.1% (95% CI 46.4–57.4%) for the 2012–2014 prevalence.

Adolescent BMI, including values within the currently accepted 'normal' range, strongly predicts DM mortality up to the seventh decade. The increasing prevalence of childhood and adolescent overweight and obesity points to a substantially increased future adult DM burden.

In light of the worldwide increase in childhood obesity, the association between BMI in late adolescence and death from cardiovascular causes in adulthood was also investigated in the Israeli subjects. Primary outcomes were the number of deaths attributed to coronary heart disease, stroke, sudden death from an unknown cause or a combination of all three categories (total cardiovascular causes) by mid-2011. During 42,297,007 person years of follow-up, 2,918 of 32,127 deaths (9.1%) were from cardiovascular causes, including 1,497 from coronary heart disease, 528 from stroke and 893 from sudden death. On multivariable analysis, there was a graded increase in the risk of death from cardiovascular causes and all causes that started among participants in the group that was in the 50th–74th centiles of BMI. HRs in the obese group (≥95th centile for BMI) compared with the reference group in the 5th–24th centiles were 4.9 (95% confidence interval [CI], 3.9–6.1) for death from coronary heart disease, 2.6 (95% CI, 1.7–4.1) for death from stroke, 2.1 (95% CI, 1.5–2.9) for sudden death and 3.5 (95% CI, 2.9-4.1) for death from total cardiovascular causes, after adjustment for sex, age, birth year, sociodemographic characteristics and height. HRs for death from cardiovascular causes in the same centile groups increased from 2.0 (95% CI, 1.1–3.9) during follow-up for 0–10 years to 4.1 (95% CI, 3.1–5.4) during follow-up for 30–40 years; during both periods, HRs were consistently high for death from coronary heart disease. Findings persisted in extensive sensitivity analyses: a BMI in the 50th–74th centiles during adolescence, i.e. within the accepted normal range, was associated with

increased cardiovascular and all-cause mortality during 40 years of follow-up. Overweight and obesity were strongly associated with increased cardiovascular mortality in adulthood.

It has been hypothesized that childhood obesity may be associated with mortality in a pediatric intensive care setting (PICU). Using a large multicentre PICU database, the association between obesity and PICU mortality, adjusting for initial severity of illness, was assessed. Whether or not height- and weight-based classifications of obesity compared with a weight-based classification alone may alter the mortality distribution was investigated. Height, weight, age and gender were used to calculate z score groups based on Centers for Disease Control and Prevention and World Health Organization growth curves. A random effects-mixed logistic regression model was used to evaluate the association between obesity and PICU mortality, controlling for hospital, initial severity of illness and co-morbidities.

A total of 127,607 patients could be included; the mortality rate was 2.48%. Being overweight was independently associated with increased PICU mortality after controlling for severity of illness with the pediatric index of mortality 2 score and pre-existing co-morbidities. Mortality had a U-shaped distribution when classified according to weight for age or weight for height/BMI. When classifying patients using weight for age without respect to height, the nadir of the mortality curve was shifted, potentially falsely implying a benefit to mild obesity.

Risk-adjusted PICU mortality significantly increases as weight for height/BMI increases into the overweight and obese ranges. It is believed that height data are necessary to correctly classify body habitus; without such information, a protective benefit from mild obesity may be incorrectly concluded or even a negative effect of obesity may become obscured.

Health Economics

The financial burden of childhood obesity for industrialized societies can only be estimated. The annual economic costs due to medical expenses and lost income as a result of complications of adult obesity are ~$70 billion in the USA. At least another $30 billion are thought to be spent on diet foods, products and programmes to lose weight. If one is to calculate prospective costs of obesity that has started at an early age, the costs are even higher. On the other hand, it is almost too ironic to state that sales and profits of the obesity treatment industry have already reached an enormous sum. Obesity in childhood and adolescence has already become a major factor in healthcare planning systems and within the healthcare industry. In addition to the prospect of diminished health, obese people are often stigmatized socially and in the workplace that contributes to the economic cost of obesity, albeit in an unknown and almost incalculable way.

Treatments

Medical Management

Any meta-analysis on the efficacy of treatment and even more so on the prevention of overweight and obesity at a young age has failed to show any large effects of treatment. Barriers to participate and obstacles to access treatment and prevention programmes are one reason, but families with an obese child often have few educational and socioeconomic resources and will sometimes completely fail to understand the importance and/or relevance of treatment. The affected child will frequently have obese family members whose obesogenic environment and lifestyle influence the child's environment and lifestyle. Since in many cases the co-morbidities of obesity are not felt at a young age, there is little individual and personal incentive to change habits and/or unhealthy lifestyles. Lastly, if one is to live with obese peers, the motivation to lose weight or change one's unhealthy behaviour will be very limited indeed.

Multidisciplinary Therapeutic Approach

Because obesity is a risk factor for so many co-morbidities, effective treatments need to be developed and made widely available. Therapeutic strategies include psychological and family therapy intervention, lifestyle/behaviour modification and nutritional education. The role of regular exercise and exercise programmes should be emphasized. Intermittent exercise (high intensity followed by low intensity sports) may result in a greater reduction in weight and body fat mass. Such approaches increase compliance/adherence rates in the younger cohort of obese individuals.

Multidisciplinary outpatient treatments are considered to be the most effective strategy. In most countries, networking of primary care physicians, public health/school medicine institutions, specialists of pediatric and adolescent medicine, social workers, child psychologists and dieticians, as well as sport educators, could be achieved. Health insurance providers and policymakers should strongly support such networking concepts. Using such approaches, some workers have reported high success rates and sufficient long-term weight reductions in small groups of children studied, but data from large, randomized, targeted trials are few.

Nutrition Treatment and Dieting

Repeated dieting with moderate weight loss and gain does not lead to serious weight loss and sustained weight maintenance and may be harmful both in respect to higher weight in the end and increasing the likelihood of co-morbidities. Reducing high fructose and high sucrose intake does maintain a healthy body weight so reducing the consumption of sugar-sweetened soft drinks is thought to be useful. Laws and regulations to reduce the marketing of such drinks have been introduced in many countries but have been defeated by food industries that have the power and financial resources to counteract any wise suggestions from scientists and physicians.

Snacking is associated with the development of obesity both in individuals and societies, so to organize food intake in infancy and at school age in a way that breakfast, lunch and dinner with a small snack of fruit or vegetable in the morning and afternoon are eaten with the family or community helps to structure the day and keeps snacking at bay.

Physical Activity Training

To lose weight, a substantial amount of physical activity must be started and maintained. Recommendations for school age children are available in many countries and societies, but it has to be made clear that daily physical activity with walking or bicycling to school and taking stairs instead of elevators to ascend to upper floors does provide useful physical activity in addition to sports sessions at school. Using physical activity training as a means to lose weight requires structured, heavy duty physical training under the supervision of personal trainers or teachers and has to be maintained over a long period of time. Not many families nor societies can guarantee this for a large number of children and adolescents.

Behavioural Therapies

Environmental approaches to obesity prevention aiming for multidimensional interventions may be effective in reducing the number of obese individuals in a society but no single behavioural or environmental intervention can be effective on its own. There have been numerous attempts to identify the optimal psychological intervention and behavioural modification techniques to treat obesity, but no behavioural therapy can intervene with the environmental, societal, sociodemographic, genetic and biologic causes of childhood obesity, so it is not surprising that all such attempts have been unsuccessful. Barriers that prevent families and obese children participating in behavioural and multidisciplinary treatment programmes are neither identified nor understood, but small-scale experimental studies of psychological techniques have been developed that help to encourage young patients and their families to adopt lifestyle modifications and lead healthy lives.

Family-Based Therapies and Community-Based Therapies

Approaches targeting both the family and the community are the most promising strategies for treatment and prevention. Individuals need peer or family support to change an obesogenic lifestyle but mixed and multiprofessional and interdisciplinary approaches to treat childhood obesity are cumbersome, costly and only marginally successful with changes in BMI SDS of no more than –0.2 being seen in most studies. If parents and siblings are involved and participate in the obese child's treatment, success rates are higher but poor income and low education remain major obstacles.

Bariatric Surgery

Bariatric surgery has become an effective option as a last ditch treatment for severe obesity in adults, and bariatric interventions are now the most commonly undertaken surgical procedures in the USA. In adolescents and young adults, data on safety and efficacy are few. In a prospective longitudinal registry that has been operative since January 2005, patients undergoing bariatric surgery in Germany are enlisted in a 'study for quality assurance in obesity surgeries'. Among the patients registered, there are 345 adolescents and young adults up to the age of 21 years who underwent surgery between January 2005 and December 2010 in a total of 58 hospitals, but only 51 patients were under the age of 18 years. Follow-up information is available for 48% (n = 167) of patients, with an average observation period of 544 ± 412 days (median: 388 days).

The most common techniques were gastric banding (n = 118, 34.2%), gastric bypass (n = 116, 33.6%) and sleeve gastrectomy (n = 78, 22.6%). Complications were considered to be low but are relevant: short-term complications were slightly lower for gastric banding (0.8–8.0%) than for gastric bypass (1.7–5.2%) or sleeve gastrectomy (7.7–9.0%). Weight and BMI reduction were lower for gastric banding (–28 kg; –9.5 kg/m^2) compared with gastric bypass (–50 kg; –16.4 kg/m^2) ($P < 0.001$) or sleeve gastrectomy (–46 kg; –15.4 kg/m^2) ($P < 0.001$). Outcomes did not differ between the <18 and ≥18-year-old patients. A final conclusion about lasting efficacy and safety is wanting at present.

Laparoscopic adjustable gastric banding is increasingly considered to be the treatment of choice in very obese adults. Early complications of such interventions and significant late complications such as pouch dilatation

and stomach slippage have been rare but, in one series, re-operations were necessary in 7.5% of 146 cases operated on. Recommendations of an international workshop on gastric banding for adult obesity suggested that (1) careful patient selection has to be made, (2) standard surgical practice has to be adhered to and (3) no surgery must be performed without the support of an interdisciplinary team that includes internists, psychologists and dieticians. Whether such invasive treatment options will be considered in children is open to debate. There is currently no experience with the procedure in children.

Pharmacotherapy

Long-term treatment also including extended pharmacotherapy may be necessary for the majority of very obese adolescents. Table 17.6 lists some drugs used in obesity management in adults. At present Orlistat is increasingly used in obese adults. Orlistat binds to gastrointestinal lipases and causes a partial inhibition of fat reabsorption from the gut. When combined with a hypocaloric diet, it leads to a moderate additional weight loss of some kilograms within 6 months, but great care should be exerted when anti-obesity medication is to be prescribed to children. Several previously widely used medications have been withdrawn from the market because of concerns about side effects in adults. Few if any of these drugs have been studied in respect to efficacy, safety and long-term outcomes in children and adolescents, and it is of concern that some of these drugs are being prescribed to youngsters by primary care physicians upon parental request without scientific follow-up studies and without careful and systematic follow-up.

Table 17.6 Drugs that could potentially be used in obesity management in children and adolescents.

Drugs approved for use in adult obesity in some countries at some time:
Sibutramine
Phentermine
Mazindol
Diethylpropion
Orlistat
Drugs under development:
Leptin and leptin analogues
Brain and gut peptide agonists or antagonists
MC4-receptor peptide agonists
NPY-Y1 or – Y5 antagonists
Galanin receptor antagonists
Orexin receptor antagonists
Alpha1-receptor agonists
Beta2-receptor agonists

Note: most of these are not recommended/licenced for use in children.

Children with type 2 diabetes who are acutely ill are treated with insulin and some will later change to oral antidiabetic agents, the safety and efficacy of which have not been established for children and adolescents. The only drug currently approved for therapy of children with diabetes is insulin. Even less is known about therapy in children with co-morbid conditions that frequently accompany type 2 DM. There are no guidelines for what therapy to use or when to employ it, for such important states as hyperlipidaemia and hypertension, and recommendations for combination therapies in children are being considered.

Prevention

Because treatment is so difficult, prevention of obesity is important and needs to focus not just on individual characteristics when planning or implementing interventions. Complex problems need complex solutions and, since the reasons for obesity are multidimensional, multidimensional strategies focusing on the individual, the family and the immediate and wider environments are needed (Table 17.7).

Environmental changes are possible, effective and sustainable. Individual or family characteristics such as socioeconomic position, family habits and health behaviour are more difficult to change in the long term by person-based educational programmes. Changes at the environmental level (e.g. food supply in schools, more physical activity) are relatively easy to apply and have the potential to change individual behaviours as well as wider attitudes.

Environmental or community approaches may reach those who are really in need. Traditional interventions that focus on individual behaviours have failed to reach the population groups at the highest risk (individuals

Table 17.7 Preventive strategies targeting overweight and obese children and adolescents.

Generally, interventions may operate at different levels (Whitehead 2007):
• Strengthening individuals using person-based educational approaches
• Strengthening communities by building social cohesion and mutual support
• Improving living and working conditions by reducing exposure to health damaging environments and improving conditions for healthy environments
• Promoting healthy macro policies

with low SES). Thus, over the years, there has been a shift from person-based intervention strategies to community-based approaches.

The easiest way to change an individual's living environment is to move to another neighbourhood. A large-scale social experiment 'moving to opportunity', conducted in the cities of Baltimore, Boston, Chicago, Los Angeles and New York, was published in 2011. The development of body weight and glycated haemoglobin of 1788 women who were randomly given the opportunity to move with their children from public housing in a high poverty area to a low poverty area was studied. 10–15 years after randomization, a lower prevalence for overweight and elevated concentrations of glycated haemoglobin (HbA1c) in the intervention group was noted. The authors were not able to explain the mechanisms underlying these effects, but this study shows the public health potential of modifying environmental factors for health improvement.

As prevention has to start very early in life, perhaps even before postnatal life, a population and community approach for prevention seems to be promising and reasonable, but primary prevention has proven difficult or impossible in most societies. Again, a multidisciplinary team approach aims to develop and secure preventive strategies.

Good nutrition and modest exercise for pregnant women as well as monitoring of intrauterine growth of the child are mandatory. After birth, rapid weight gain should be avoided and principles of good nutrition and physical activity should be taught at all ages. Breastfeeding should be strongly recommended. One study has found that the prevalence of obesity in children who had never been breastfed was 4.5% as compared with 2.8% in breastfed children.

Early intervention and guidance can influence children's food choices. Parents should be encouraged to make healthy foods easily available to the child and serve these foods in positive mealtime situations in order to help their child to develop healthy food habits. As is the case for treatment strategies, multidisciplinary teams are needed. They should include a physician, a nutrition specialist and a psychologist but consist mainly of school nurses, teachers and kindergarten teachers. Joint actions by physicians, health authorities and politicians both in the community and using modern and social media are required to implement nationwide prevention programmes that take cultural and racial preferences and attitudes in respect to food preparation and eating habits into account. The food industry must face up to its responsibilities and stop marketing unhealthy foods and advertising these to children and adolescents.

Community and Society

Obesogenic environments are the sum of influences that the surroundings, opportunities or conditions of life have on promoting obesity in individuals or populations. They comprise a resident's aggregate SES as well as aspects of their social and physical environments. Evidence suggests that children living in low SES areas are more often overweight or obese. Aspects of the social environment (social cohesion, collective socialization and trust) seem to be relevant for unhealthy weight gain in children and adolescents.

Regarding the physical environment, three domains that may influence obesity include (1) facilities for physical activity (i.e. parks, playgrounds, sports clubs that promote active play and sports), (2) land use and transportation (i.e. mixed land use, walkability, access to public transport or walking/cycling paths that facilitate active commuting to school/work) and (3) foodscape (availability of healthy or unhealthy food). Fewer resources for recreation (e.g. parks, playgrounds), sports or active commuting (street connectivity, land use–mix) and a high density of fast food outlets are related to overweight and obesity in children and adolescents.

Recent studies also suggest that air pollution may cause higher mortality among individuals with type 2 diabetes, increase the risk for insulin resistance in children and increase the risk for asthma in children, especially in the overweight. However, it is possible to show that children living closer to parks and recreational spaces are less likely to experience weight gain. Moreover, the availability of appropriate food outlets in a neighbourhood is not necessarily associated with unhealthy diets, but can be associated with higher consumption of fruit.

Microbiota

The potential role of intestinal microbiota in the aetiology of many human diseases has attracted a lot of attention. The intestinal microbiota has been suggested to be an important factor for the development of obesity and obesity-related metabolic dysfunction. The distal human intestine represents an anaerobic bioreactor programmed with an enormous population of bacteria, dominated by relatively few strains that are very diverse at the strain/subspecies level. This microbiota and its collective genomes (microbiome) provide us with genetic and metabolic traits that we have not been required to develop on our own, including the ability to digest and utilize otherwise inaccessible nutrients. New studies

have revealed how the gut microbiota has coevolved with the human species and how it manipulates and complements human biology in ways that are mutually beneficial. It is now partially understood how certain members of the microbiota function so as to maintain the stability and functional adaptability of the microbial organ. Experiments in animal models have produced evidence for a causal role of intestinal microbiota in the aetiology of obesity and insulin resistance but, with a few exceptions, such causal relation is still lacking for humans. Until now, most reports merely describe associations between the intestinal microbial composition and metabolic disorders such as obesity and type 2 diabetes in the human. Thus, the reciprocal relation between the bacteria and these metabolic disorders remains a matter of debate.

The intestinal microbiota influences metabolic, nutritional and immunological processes and has been associated with a broad range of adverse health outcomes including asthma, obesity and type 2 diabetes. Early-life exposures may alter the course of gut microbial colonization leading to differences in metabolic and immune regulation throughout life. Although, for example, ~50% of low-risk full-term infants born in Canada are exposed to intrapartum antibiotics, little is known about the influence of this common prophylactic treatment on the developing neonatal intestinal microbiota. In a pivotal study the intestinal microbiome over the first 3 years of life among healthy breastfed infants born to women with low-risk pregnancies at full-term gestation has been analysed to determine if, at 1 year of age, the intestinal microbiome of infants exposed to intrapartum antibiotics differs in type and quantity from the infants that are not exposed.

A prospectively followed cohort of 240 mother–infant pairs was formed in Ontario, Canada. Participants were followed until the age of 3 years. Study questionnaires were completed, anthropometric measures taken and biological samples obtained including eight infant stool samples between 3 days and 3 years of age. It was clearly shown that early antibiotic use will have a long-lasting effect upon the development of the infant's microbiota. Experience also indicates that midwifery client populations are likely to have high rates of breastfeeding and low rates of intervention, allowing one to examine the comparative development of the microbiome in a relatively healthy and homogenous population.

In terms of early obesogenic signals, it seems that an intestinal microbiome signature persists after successful dieting of obese mice, which contributes to faster weight regain and metabolic aberrations upon re-exposure to obesity-promoting conditions and transmits the accelerated weight regain phenotype upon inter-animal transfer. In animal studies, it was also found that the microbiome contributes to diminished post-dieting flavonoid concentrations and reduced energy expenditure. This finding might demonstrate that flavonoid-based 'post-biotic' intervention could potentially ameliorate excessive secondary weight gain. Such data highlight a possible microbiome contribution to the development of weight gain and an accelerated post-dieting weight regain. This might suggest that microbiome-targeting approaches may help to prevent and even treat obesity in early life.

Urban Living

Access to playgrounds, restrictions on food marketing for children, low pricing of healthy foods and changes in food supplies in cafeterias, walkability of living quarters and the planning of cities all should be considered if one is seriously to prevent childhood obesity on a large scale. Green areas should be interspersed in living quarters and free access to pedestrian zones should be ensured. There is a large quantity of research that indicates how cities should be developed to support healthy lifestyles, especially in the young, but since, in most countries, there is no legislation that secures such developments, most city dwellings are developed following financial incentives of investors rather than scientific reasoning.

Advertising Industries

In some countries, advertisements targeting children and youngsters are already forbidden and may be punished by law, but there is still food advertising targeting children in many areas of the world. In many instances, large sums of money are invested by food industries to invent the most clever and attractive way to seduce children. Sugar-sweetened beverages are but one example of how children are falsely educated to consume too many calories and too much carbohydrate. In addition, food industries are good at diluting the evidence that, indeed, sugar-rich beverages and foods are unhealthy and contribute to the obesity epidemic by placing advertisements in physicians' journals and other media. Bans of advertising for tobacco have shown the way forward for the food industry; after tobacco advertising was banned on a large scale and cigarettes taxed heavily, the number of smokers and the prevalence of lung cancer have declined. Taxes on

unhealthy foods, be it fat or sugar rich, have now been implemented in Denmark and the UK.

Conclusions

The amount and nature of adipose tissue of a child at which morbidity acutely and/or later in life increases is determined on an actuarial basis. Direct measurements of body fat, for example, hydrodensitometry, bioimpedance or DEXA, are useful only in a research context. BMI is easy to calculate and widely accepted to define obesity in children and adolescents clinically and for practical purposes. An increased risk of death from cardiovascular disease in adults has been found in subjects whose BMI had been greater than the 75th centile as adolescents. Childhood obesity particularly increases the risk of subsequent morbidity, whether obesity persists into adulthood or not (Tables 17.8–17.10).

The genetic basis of childhood obesity has been elucidated through the discovery of leptin and the increasing knowledge on the role of neuropeptides such as POMC, NPY and the melanocyte-stimulating hormone receptors (for example, MC4R) and the discovery of FTO as an obesity risk allele. However, environmental/exogenous factors largely contribute to the development of a high degree of body fatness early in life. Twin studies suggest that ~50% of the tendency towards obesity is inherited while 50% is related to socioeconomic and lifestyle factors.

Numerous disorders including endocrine disorders (Cushing syndrome, hypothyroidism etc.) and genetic syndromes (Prader–Willi syndrome, Cohen and Alström syndrome, Bardet–Biedl syndrome) are associated with an increased body fat mass. Usually, a simple clinical diagnostic algorithm allows the differentiation between primary and secondary obesity.

The most common sequelae of primary childhood obesity are hypertension, dyslipidaemia, impaired insulin sensitivity, back pain and psychosocial problems. Therapeutic strategies include psychological and family therapy, lifestyle/behaviour modification and nutritional education. The role of regular exercise and exercise programmes should be emphasized. Surgical procedures and drugs used in adult obesity are not generally recommended for obese children and adolescents.

As obesity is the most common chronic disorder in industrialized societies, its impact on individual lives as well as on health economics should be more widely recognized and public awareness of the increasing health burden and economic dimension of childhood obesity aroused. Efforts to investigate prevention and interventions that will work both on an individual and at a societal level need to be reinforced.

Table 17.8 Unresolved questions in obesity research.

Why do people continue to eat beyond their physiological need?
Why do people eat unhealthy foods and do not exercise regularly?
Why do people not adhere to healthy life style rules?
Why do people not participate in obesity programmes?
Why do many people not take their medications?

Table 17.9 Unsolved questions in relation to obesity research and clinical practice for care for children and adolescents with overweight and obesity.

Organization and aims of inpatient care
Organization and aims of outpatient care
Community healthcare delivery
Questions of all aspects of prevention
Clinical relevance, affordability, cost–benefit ratio of diagnostic tools
Behavioural science questions
Societal, cultural and socioeconomic impact
Barriers to participate
Psychology, psychobiology
Nursing
Management
Quality of life
Humanities
Policy and politics involvement

Table 17.10 Summary of multiple determinants of childhood overweight and obesity.

Policy
Norms
Culture
Society
Walkability
Recreational/sports facilities
Air pollution
Neighbourhood
Foodscape
Social norms
Social capital
Food
Physical activity
Health
Behaviour
Socioeconomic status (SES) child
Genes
Family
School/Kindergarten

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Weblinks

http://easo.org/task-forces/childhood-obesity-cotf/

www.iaso.org

18

Genetic Obesity Syndromes

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KEY LEARNING POINTS

- Childhood onset obesity is highly heritable.
- Genetic obesity syndromes currently explain 10% of children with severe obesity.
- All genetic obesity syndromes known to date lead to increased appetite and food-seeking behaviours.
- Some genetic disorders affect basal metabolic rate in addition to effects on appetite.
- Leptin deficiency can be treated effectively with recombinant leptin.
- The recent availability of a melanocortin 4 receptor agonist offers promise for the treatment of some obesity syndromes such as POMC deficiency.

Introduction

The rising prevalence of childhood obesity is driven by factors that promote an increase in energy intake (an abundance of inexpensive, easily available, energy-rich, highly palatable foods) and factors that contribute to a decrease in energy expenditure such as sedentary lifestyles (television watching) and reduced physical activity at school and in leisure time. However, within this obesogenic environment, there is considerable variation in body weight and fat mass between individuals, with some children much more likely to gain weight than others. This variability is influenced by complex interactions between environmental and biological (genetic, developmental and behavioural) factors that influence where an individual lies on the BMI distribution [1]. As well as an increase in the mean BMI in many populations, the proportion of children at the upper end of the distribution, those with severe obesity, has also increased [2, 3]. As the number of children presenting for clinical assessment is increasing, pediatricians need a systematic approach to the assessment of severe childhood-onset obesity and to consider genetic causes.

Genetic Contributors to Obesity: The Evidence

Evidence for the genetic contribution to body weight comes from twin studies that have informed estimates of heritability, defined as the proportion of total phenotypic variance attributable to genetic variation in a specified environment. Many twin studies have concluded that the heritability of body mass index (BMI) is between 0.71 and 0.86 [4]. A study of over 5000 UK twins aged 8–11 years growing up during a time of dramatic rises in obesity supports the substantial heritability of BMI (~77%) and showed a very modest effect of the shared environment, which can inflate heritability estimates; the remaining environmental variance was largely unshared [5].

Similar heritability estimates were found when studying identical twins reared together and apart [6] and in large studies of adopted children whose body weights tend to be similar to those of their biological parents rather than to those of their adopted parents [7]. Family-based studies generally report somewhat lower heritability estimates and are often confounded by the impact of the shared environment.

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Genetic Obesity Syndromes: An Overview

Genetic studies focussing on severe childhood obesity have led to the identification of many genes in which rare, highly penetrant variants cause obesity [8–12]. While individually these disorders are rare, cumulatively at least 10% of children with severe obesity have rare chromosomal abnormalities and/or highly penetrant genetic mutations that drive their obesity [13]. This figure is likely to increase with wider accessibility to genetic testing in clinical practice and as new genes are identified from exome and genome sequencing.

Some genetic obesity syndromes are associated with learning difficulties and developmental delay (PWS) and major clinical problems, which means that children come to medical attention at a young age, but there has been increasing recognition over the last 15 years that there is a large and increasing group of genetic disorders where severe obesity itself is the presenting feature [14, 15]. These children are often identified by early and marked weight gain, but the lack of other clinical features means that a genetic diagnosis is not considered in many and may be offered only when they reach secondary care.

The assessment of severely obese children and adults should be directed at screening for potentially treatable endocrine and neurological conditions and identifying genetic conditions so that appropriate genetic counselling and treatment can be instituted (Figure 18.1). A family history to identify potential consanguineous relationships, the presence of other family members with severe early-onset obesity and the ethnic and geographical origin of family members should be taken. The history and examination can then guide the appropriate use of diagnostic tests. For the purposes of clinical assessment, it remains useful to categorize the genetic obesity syndromes as those with dysmorphism and/or developmental delay and those without these features, but the spectrum of clinical features can be quite variable.

Obesity with Developmental Delay

Prader–Willi Syndrome

PWS (prevalence of about 1 in 25,000) is caused by deletion of a critical segment on the paternally inherited copy of chromosome 15q11.2–q12 or loss of the entire paternal chromosome 15 with 2 maternal copies

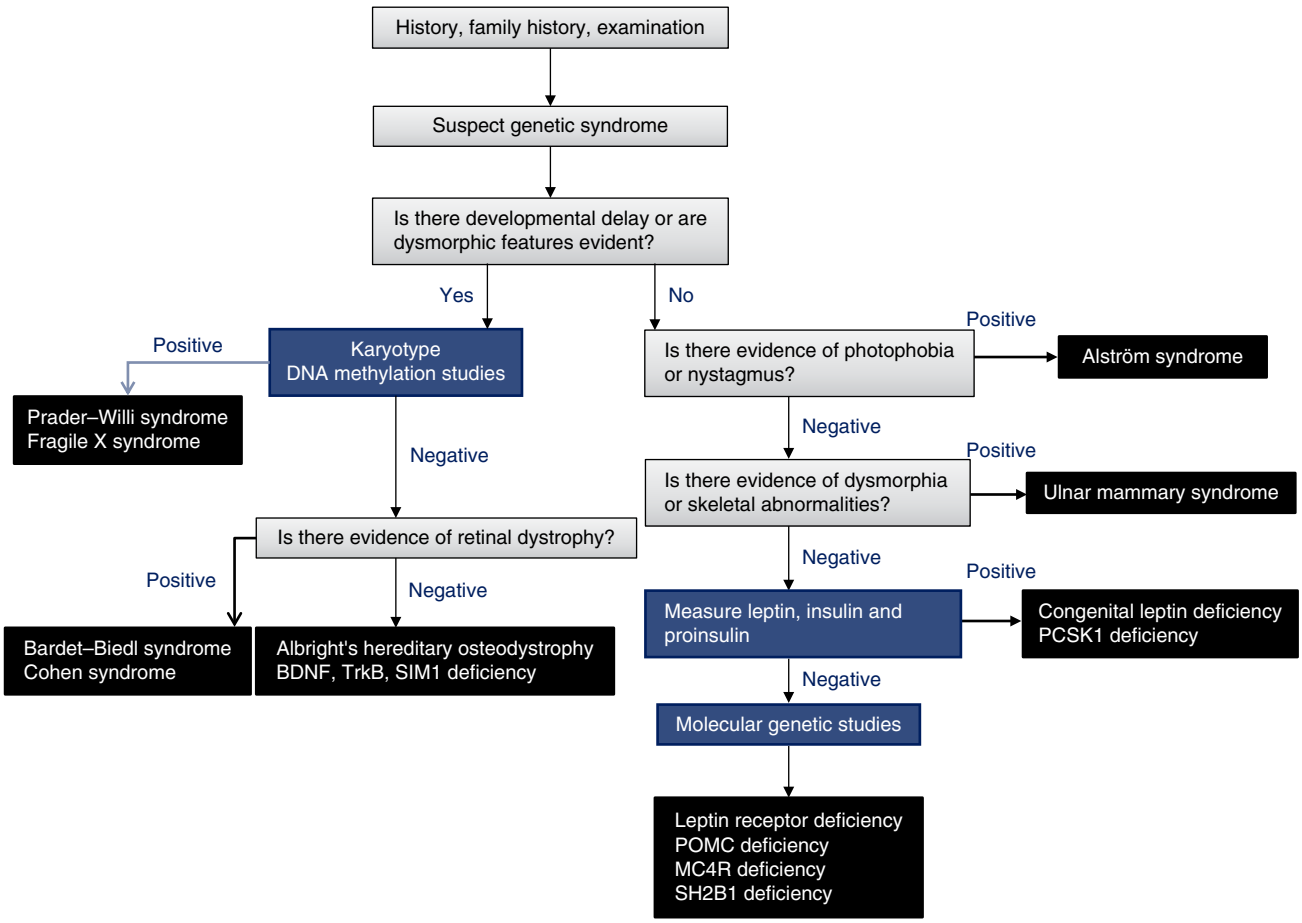


Figure 18.1 A diagnostic approach to genetic obesity syndromes.

(maternal uniparental disomy). Key features include hypotonia and failure to thrive in infancy, learning difficulties, short stature, hyperphagic obesity and hypogonadotropic hypogonadism [16]. Children with PWS have reduced lean body mass and increased fat mass, abnormalities that resemble those seen in growth hormone (GH) deficiency; GH treatment decreases body fat and increases linear growth, muscle mass, fat oxidation and energy expenditure [17, 18].

Plasma concentrations of the stomach-derived hormone ghrelin are markedly elevated in children and adults with PWS compared with obese controls and patients with other genetic obesity syndromes. The significance of this finding and its possible role in the pathogenesis of hyperphagia in these patients is unknown.

Most chromosomal abnormalities in PWS occur sporadically and can be identified by a routine karyotype. There are distinct differences in DNA methylation of the parental alleles, and DNA methylation is a reliable postnatal diagnostic tool. Small deletions encompassing only the HBII-85 family of snoRNAs have been reported in association with the cardinal features of PWS including obesity [19, 20], suggesting that these noncoding RNAs and the genes that they regulate may be important in the aetiology of PWS.

Albright's Hereditary Osteodystrophy

GNAS1 is the gene encoding Gαs protein that mediates signalling by multiple G-protein-coupled receptors (GPCRs). Imprinting at this locus results in a variable clinical phenotype in patients who carry loss of function mutations in *GNAS1*, which is classically associated with Albright's hereditary osteodystrophy (AHO). Maternal transmission of *GNAS1* mutations leads to classical AHO (short stature, obesity, skeletal defects and impaired olfaction) with resistance to several hormones (e.g. parathyroid hormone) that activate Gαs in their target tissues (pseudohypoparathyroidism type IA), while paternal transmission leads only to AHO (pseudopseudohypoparathyroidism).

Some patients with *GNAS1* mutations present with obesity without all the features. *GNAS1* is imprinted in a tissue-specific manner, being expressed primarily from the maternal allele in some tissues and biallelically in other tissues; thus multi-hormone resistance occurs only when Gαs mutations are inherited maternally [21].

Bardet–Biedl Syndrome

Bardet–Biedl syndrome (BBS) is a rare (prevalence <1/100,000) autosomal recessive disease characterized by obesity, learning disability, dysmorphic extremities (syndactyly, brachydactyly or polydactyly), retinal dystrophy or pigmentary retinopathy, hypogonadism and structural abnormalities of the kidney or functional renal impairment. BBS is a genetically heterogeneous disorder known to map to at least sixteen loci with mutations in more than one locus sometimes required for complete expression of the phenotype.

Many BBS genes appear to affect proteins localized to the basal body, a key element of the monocilium thought to be important for intercellular sensing in mammalian cells including neurons [22]. Other disorders of ciliary function such as Alström syndrome (retinal dystrophy, severe insulin resistance, deafness) and Carpenter syndrome are also associated with obesity. The presence of cilia on leptin-responsive neurons involved in energy balance and animal data suggesting that their disruption causes obesity both suggest that there is a link between ciliary function, leptin signalling and energy homeostasis that remains to be explored [23].

BDNF and TrkB Deficiency

A small number of children with severe hyperphagia and obesity, impaired short-term memory, hyperactivity and learning disability who have mutations or chromosomal deletions that disrupt brain-derived neurotrophic factor (BDNF) or its tyrosine kinase receptor tropomyosin-related kinase B (TrkB) [24] have been reported. Deletions on chromosome 11p.12 that encompass the BDNF locus are associated with early-onset obesity [25]. Given the severe developmental phenotype of these patients, it is not surprising that mutations seem to arise *de novo* and as such should be considered where both parents are of normal weight and IQ.

SIM1 Deficiency

Chromosomal deletions that disrupt single-minded 1 (SIM1) and heterozygous loss of function mutations in the gene itself result in severe obesity inherited in a dominant manner with variable penetrance [10]. SIM1 is a basic helix-loop-helix transcription factor involved in the development and function of the paraventricular nucleus of the hypothalamus and may be involved in melanocortin and oxytocin signalling. SIM1 deficient patients are hyperphagic with evidence of autonomic dysfunction (characterized by low systolic blood pressure) as seen in melanocortin 4 receptor (MC4R) deficiency as well as speech and language delay and neurobehavioural abnormalities including autistic-type behaviours. These features are not recognized features of MC4R deficiency but show some overlap with the behavioural phenotypes seen in PWS. As the hyperphagia of *sim1* haploinsufficient mice is partly ameliorated by the central administration of oxytocin [26], a neurotransmitter involved in the modulation of emotion and social interaction, impaired oxytocinergic signalling is one possible mechanism implicated in the obesity and behavioural phenotype seen in SIM1 deficiency.

Obesity Without Development Delay

Severe childhood-onset obesity can result from genetic defects involving the leptin–melanocortin pathway. Leptin is an adipocyte-derived hormone whose circulating concentrations correlate closely with fat mass and which signals through the long isoform of the leptin receptor to regulate energy homeostasis (Figure 18.2). Leptin stimulates the expression of pro-opiomelanocortin (POMC) in the arcuate nucleus of the hypothalamus, which is post-translationally modified to generate the melanocortin peptides (ACTH and alpha, beta, gamma melanocyte-stimulating hormone [MSH]), which activate melanocortin receptors in the skin (melanocortin 1 receptor [MC1R]) to modulate pigmentation, in the adrenal gland (MC2R) to regulate glucocorticoid synthesis and in the brain (MC3R and MC4R) to reduce energy intake and increase expenditure. In parallel, leptin inhibits pathways that stimulate food intake (orexigenic), effects that are mediated by neurons expressing the melanocortin antagonist Agouti-related protein (AgRP) and neuropeptide Y (NPY). These two sets of primary leptin-responsive neurons project to second-order neurons expressing the MC4R in the paraventricular nucleus of the hypothalamus and other brain regions and form a critical circuit that regulates human energy balance.

Leptin and Leptin Receptor Deficiency

Homozygous frameshift, nonsense and missense mutations in the genes encoding leptin and the leptin receptor cause recessively inherited severe obesity. Leptin receptor mutations have been found in some non-consanguineous families, where both parents are unrelated but happen to carry rare alleles in heterozygous form.

Patients are born of normal birth weight but exhibit rapid weight gain in the first few months of life, resulting in severe obesity (mean body mass index standard deviation score [BMI SDS]: +5.8–7.8). Early development is usually normal. The most notable feature is intense hyperphagia with food-seeking behaviour and aggressive behaviour when food is denied. Increased food-seeking behaviour continues into later life in the adult subjects that have been reported [27].

Children with leptin deficiency have marked abnormalities of T-cell number and function [28] consistent with high rates of childhood infection and of mortality from infection [27]. In those that survive, obesity continues into adult life with hepatic steatosis [29] and hyperinsulinaemia consistent with the severity of obesity. Some adults have developed type 2 diabetes in the third to fourth decade [30].

Leptin and leptin receptor deficiency are associated with hypothalamic hypothyroidism and hypogonadotropic hypogonadism, but there is some evidence for the delayed but spontaneous onset of menses in some leptin- and leptin receptor-deficient adults. Leptin- and leptin receptor-deficient children have normal linear growth in childhood and normal IGF-1 concentrations, but final adult height is reduced due to the absence of a pubertal growth spurt.

Although leptin deficiency appears to be rare, it is treatable with daily subcutaneous injections of recombinant human leptin [28] that is currently available to

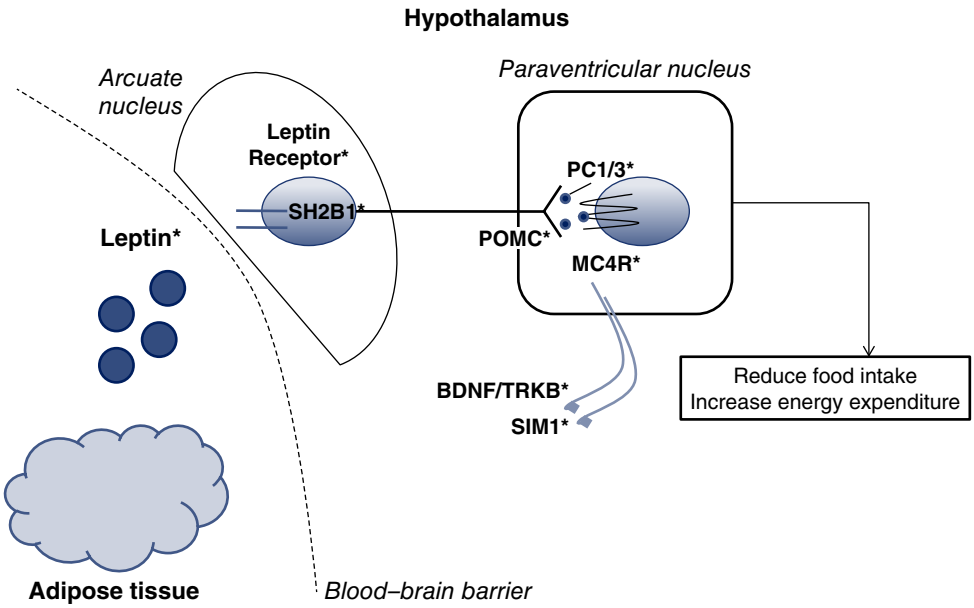
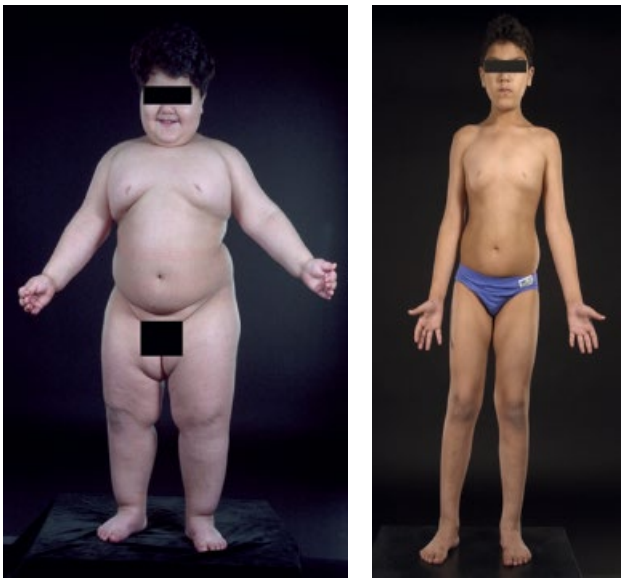


Figure 18.2 Schematic of the hypothalamic leptin–melanocortin pathway. *Indicate genetic obesity syndromes.



3-year old weighing 42 kg | 7-year old weighing 32 kg

Figure 18.3 A 3-year-old boy with congenital leptin deficiency, weighing 42 kg before (left) and 32 kg after (right) 4 years of treatment with recombinant leptin therapy.

patients on a named patient basis in a small number of centres around the world (Figure 18.3). The major effect of leptin treatment is on food intake, with normalization of hyperphagia and enhanced satiety [28, 31]. Importantly, leptin also has permissive effects on the development of puberty and if given in early childhood permits appropriate linear growth.

Serum leptin is a useful test in patients with severe early-onset obesity as an undetectable concentration is highly suggestive of congenital leptin deficiency. Very rare mutations in the *LEP* gene that result in a bioinactive form of the hormone but appropriate leptin concentrations have also been reported [32]. Serum leptin concentrations are appropriate for the degree of obesity in leptin receptor-deficient patients, and an elevated serum leptin concentration is not necessarily a predictor of leptin receptor deficiency [15]. However, some *LEPR* mutations resulting in abnormal cleavage of the extracellular domain of LEPR, which acts as a leptin binding protein, are associated with markedly elevated leptin concentrations [33].

Disorders Affecting Pro-Opiomelanocortin (POMC) and POMC Processing

Children who have homozygous or compound heterozygous mutations in the *POMC* gene present in neonatal life with adrenal crisis due to ACTH deficiency because POMC is a precursor of ACTH in the pituitary. They require long-term corticosteroid replacement [34]. Such children have pale skin, and white Caucasians often have red hair due to the lack of MSH function at MC1R in the skin. Children from different ethnic backgrounds may have a less obvious phenotype.

POMC deficiency results in hyperphagia and early-onset obesity due to loss of melanocortin signalling at the MC4R. The clinical features are comparable with those reported in patients with mutations in the receptor for POMC-derived ligands, MC4R. Selective melanocortin receptor agonists are in clinical trials and may be feasible therapies for such patients in the future [35].

PC1/3 (PCSK1) Deficiency

Proprotein convertase subtilisin/kexin type 1 and 2 (PCSK1 and PCSK2) are expressed in neuroendocrine tissues where they cleave prohormones including POMC, pro-thyrotropin-releasing hormone (TRH), proinsulin, proglucagon and progonadotropin-releasing hormone (GnRH) to release biologically active peptides. Compound heterozygous or homozygous mutations in the *PCSK1* gene, which encodes PC1/3, cause small bowel enteropathy, and patients may present in neonatal life or early infancy with persistent diarrhoea requiring parenteral feeding. Other important clinical features include hypoglycaemia and complex neuroendocrine effects (including diabetes insipidus) due to a failure to process a number of prohormones. Hyperphagia and severe obesity tend to become apparent by 2–3 years of age [11, 36]. Measurement of the ratio of immature proinsulin to mature insulin is a useful diagnostic test.

MC4R Deficiency

Heterozygous loss of function mutations in *MC4R* represents the most common genetic form of obesity, and assessment of the sequence of the MC4R is increasingly seen as a necessary part of the clinical evaluation of the severely obese child [14]. The prevalence of pathogenic *MC4R* mutations ranges from 0.5 to 2.5% of people with a BMI > 30 kg/m^2 in UK and European populations to 5% in patients with severe childhood obesity [14, 37]. Most patients have heterozygous mutations; co-dominance, with modulation of expressivity and penetrance of the phenotype, is the most appropriate descriptor for the mode of inheritance. Homozygous mutations in *MC4R* have been identified in children from consanguineous families.

The clinical features of MC4R deficiency include hyperphagia in early childhood and accelerated linear growth, which may be a consequence of disproportionate early hyperinsulinaemia [38]. Reduced sympathetic nervous system activity in MC4R-deficient patients is likely to explain the lower prevalence of hypertension

and lower systolic and diastolic blood pressures seen in adults [39]. Thus, leptin–melanocortin signalling appears to play an important role in the regulation of blood pressure and its coupling to changes in weight.

Several studies have shown that adolescents and adults with heterozygous *MC4R* mutations respond to Roux-en-Y-bypass surgery [40]. As most patients are heterozygotes with one functional allele intact, it is possible that small molecule MC4R agonists or pharmacological chaperones that improve receptor trafficking to the cell surface might be appropriate treatments for this disorder. A number of compounds are under development, and one is likely to be in phase III clinical trials in the near future (www.mc4r.org.uk).

SH2B1 Deficiency

Severe obesity without developmental delay is associated with a significantly increased burden of rare, typically singleton copy number variants (deletions/duplications) [41]. Deletion of a 220 kb segment of 16p11.2 is associated with highly penetrant familial severe early-onset obesity and severe insulin resistance [13]. This deletion includes a small number of genes, one of which is *SH2B1*, known to be involved in leptin and insulin signalling. These patients gain weight in the first years of life, with hyperphagia and fasting plasma insulin concentrations that are disproportionately elevated compared with age- and obesity-matched controls. Several mutations in the *SH2B1* gene have been reported in association with early-onset obesity, severe insulin resistance and behavioural abnormalities including aggression [42].

KSR2 deficiency

To date, most of the genetic obesity syndromes are characterized by hyperphagia as a major driver of the obesity. Heterozygous mutations in the gene encoding kinase suppressor of Ras2 (KSR2) are associated with increased food-seeking behaviour in childhood and low basal metabolic rate (BMR) in the presence of normal thyroid function. Clinical reports suggested that some carriers of *KSR2* mutations experienced marked weight loss in childhood when prescribed the anti-diabetic drug metformin (for severe insulin resistance). Further work will be needed to see if these observations can be replicated in formal clinical experimental studies and to investigate the cellular mechanisms underlying these effects.

Conclusions

The diagnosis of a genetic obesity syndrome can provide information that has diagnostic value for the family to whom genetic counselling can be provided. There is particular value in a genetic diagnosis in severe obesity that, unlike other clinical disorders, is often not recognized as a medical condition by some healthcare professionals and educators. A genetic diagnosis can help children and their families deal with the social stigma that comes with severe obesity, and in some instances, where the persistence of severe obesity despite medical advice has been considered a reason to invoke parental neglect, the making of a genetic diagnosis has prevented children from being taken into care.

A genetic diagnosis can inform management (many such patients are relatively refractory to weight loss through changes in diet and exercise) and can inform clinical decision-making regarding the use of bariatric surgery (feasible in some, high risk in others). Importantly, some genetic obesity syndromes are treatable [28, 31]. There are a number of drugs in phase Ib/II clinical trials targeted specifically at patients with genetic obesity syndromes (www.rhythmtx.com).

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Weblinks

www.goos.org.uk

www.mc4r.org.uk

19

Endocrine Care During Adolescence into Young Adulthood

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KEY LEARNING POINTS

- Endocrine care for adolescents and young adults should encompass all the elements of developmentally appropriate healthcare.
- Healthcare professionals require a comprehensive understanding of adolescent biopsychosocial development to work effectively with this age group.
- There is an emerging evidence base for transition and transfer that should be applied to endocrine services.
- The diagnosis of polycystic ovary syndrome in adolescents lacks consensus, yet addressing the young person's issues is essential.
- Asymptomatic gynaecomastia in adolescence is common, and symptomatic gynaecomastia is associated with significant psychosocial concerns.
- All patients treated with rhGH for childhood-onset GHD require retesting and discussion about the evidence for continuing with rhGH after completing linear growth.
- Oestrogen deficiency is associated with reduced life expectancy, largely due to an increase in cardiovascular disease.
- Glucocorticoid replacement in congenital adrenal hyperplasia (CAH) needs to be individualized in late adolescence to optimize control in classical CAH and potentially withdrawn in non-classical CAH.

Introduction

There is increasing focus on the healthcare of adolescents and young adults and on their transition and transfer to adult services. The biopsychosocial development associated with adolescence can both be affected by having an endocrine condition and also affect a young person's ability to engage with healthcare. Healthcare professionals need not only have a working knowledge of the diagnosis and management of endocrine conditions in adolescence and young adulthood but should also have the skills and behaviours to deliver developmentally appropriate healthcare.

Adolescence and Young Adulthood

Adolescence can be variably defined but, while the biological changes of adolescence are fairly universal, the psychological and social changes of this period may vary across time, cultures and socio-economic situations and in health and illness. The WHO defines adolescence as between ages 10 and 19 but extends this up to 24 years of age to define young people [1]. This reflects the acceptance that the developmental period extends beyond adolescence into young adulthood, sometimes described by psychologists as 'emerging adulthood' [2]. Young adults may have logical competencies similar to older adults but are exposed to different social and emotional factors and exhibit differences in making decisions and taking risks. This period of psychosocial development is mirrored by changes observed in brain development that extend up to the middle of the third decade [3].

Biopsychosocial Development and Growing Up with an Endocrine Condition

Young people with endocrine conditions are at a time of their life that is associated not only with rapid physical growth and development but also with psychosocial change. The impact of going through adolescence and having an endocrine condition is bidirectional [4]; in that, growing up with an endocrine condition may affect aspects of life other than just physical development, for example, interfering with peer relationships and educational attainment, yet progress through normal adolescence will affect

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Companion website: www.wiley.com/go/dattani/brookcpe7

Table 19.1 Biopsychosocial development through adolescence.

Aspect	Early adolescence	Middle adolescence	Late adolescence
Biological	Secondary sex characteristics Beginning of rapid growth	Growth velocity peaks Body shape and composition change Acne and odour Menarche/spermarche	Physically mature Slow growth
Cognitive and moral	Concrete operations Unable to perceive long-term outcome of current decision-making Conventional morality	Emergence of abstract thought (formal operations) May perceive future implications, but may not apply to decision-making Questioning more	Future orientated with sense of perspective Idealism Absolutism Able to think things through independently
Self-concept, self-esteem and self-efficacy	Preoccupied with changing body Self-consciousness with appearance and attractiveness Fantasy and present orientated	Concern with attractiveness Increasing introspection 'Stereotypical adolescent'	More stable body image Attractiveness may still be of concern Emancipation complete Firmer identity
Sexual	Increased interest in sexual anatomy Anxieties and questions about genital changes, size Limited dating and intimacy	Testing ability to attract partner Initiation of relationships and sexual activity Questions of sexual orientation	Consolidation of sexual identity Focus on intimacy and formation of stable relationships Planning for future and commitment
Family	Increased need for privacy Increased bid for independence	Conflicts over control and independence Struggle for acceptance of greater autonomy	Emotional and physical separation from family Increased autonomy
Peers	Seeks same-sex peer affiliation to counter instability	Intense peer-group involvement Preoccupation with peer culture Peers provide behavioural example	Peer group and values recede in importance Intimacy/possible commitment takes precedence
Education and vocation	Middle school adjustment	Gauging skills and opportunities	Career decisions (e.g. college, work)

how young people manage their condition and engage with healthcare potentially resulting in reduced engagement and consequent suboptimal endocrine management. This constitutes a major challenge for the individual, his/her family and the healthcare team. Understanding these wider aspects of adolescent development in the context of having an endocrine condition is essential to work effectively with young people. Table 19.1 describes the different aspects of adolescent development (adapted from [5]).

Biological Development

Progress through puberty, the increase in growth velocity and achievement of adult height at an appropriate time, and achievement of secondary sexual characteristics with the ability to reproduce, define normality; however, altered timing of puberty, short stature and subfertility are common in young people with endocrine conditions and can affect psychosocial development [6–8]. Delayed puberty, for example, may result in low self-esteem particularly in boys and in difficulty in separating from parents and getting work due to apparent immaturity [9]. On the other hand, early puberty in girls is linked to increased risk of early sexual activity and teenage pregnancy and poor mental health in adolescence [10–13].

Emotional Well-Being

Awareness of emotional well-being and mental health issues in adolescence is paramount. Around 75% of lifetime mental health disorders have their onset before 18 years of age, with the peak onset of most conditions being from 8 to 15 years. Approximately 10% of adolescents suffer from a mental health problem at any one time [14]. Childhood long-term conditions are associated with a greater vulnerability for emotional problems (i.e. depression and anxiety) in childhood and adolescence and also beyond in adult life and young people with endocrine conditions are no exception [15].

Cognitive Development

The key cognitive development of adolescence is to move from concrete operations, when young people are unable

to perceive long-term outcomes of decision-making, to the emergence of abstract thought, also called formal operational, when a young person may perceive future implications although will not initially be able to apply it to decision-making. Knopf et al. [16] reported that a third of adolescents with a long-term condition were still at the concrete operational stage of cognitive development and a further third were in a transitional stage and not yet at the more adult abstract stage.

Although some young people with endocrine conditions have difficulties in cognition due to an underlying congenital/genetic condition or treatment such as radiotherapy, others will not. Regardless, the stage of cognitive development needs to be taken into consideration, particularly when considering the young person's level of engagement with healthcare. For example, poor adherence in a young person with endocrine conditions may be because of poorly developed abstract thinking, which may manifest as a reduced ability to plan and prepare for different situations using abstract concepts because of an immature ability to imagine future consequences and a concept of themselves as 'bullet proof' [4]. An earlier stage of cognitive development will mean that the prevention of long-term complications may not motivate a young person who has poor adherence to improve compliance.

Self-Concept, Self-Esteem and Self-Efficacy

Adolescents develop their self-concept that influences, and is in turn influenced by, self-esteem and self-efficacy. Self-concept refers to the overall idea of who a person thinks he or she is, whereas self-esteem refers to the judgements and evaluations a person makes about their own self-concept. Self-efficacy refers to the person's belief in their ability to perform a task within a specific context. Accordingly, concerns about self-concept can influence self-esteem and self-efficacy, and belief about self-efficacy can influence self-esteem, which influences self-concept. There is a risk that self-concept, self-esteem and self-efficacy may be affected in young people with long-term conditions, which may influence communication and engagement with healthcare as well as wider social development and emotional well-being. Body image and sense of attractiveness are aspects of self-concept; a meta-analysis showed that adolescents with a range of long-term conditions report higher body dissatisfaction than healthy adolescents [17].

Family

Reducing dependency on parents and other adults is a key part of adolescent development that could be compromised by having a long-term condition resulting in adolescents relying on parents for support. Conversely, parents can be overprotective and fail to help adolescents take responsibility for managing their own health needs. Adolescents who reported living with long-term conditions reported poor psychosocial health and greater difficulties with interparental and adolescent–parent relationships compared with healthy peers [18]. In addition, having a child with a long-term condition has been reported variably as affecting family functioning [4].

Peers

Peer relationships are important to children and become increasingly important as the child moves through adolescence into adulthood [19].

A recent meta-analysis compared the social functioning of youth with and without long-term conditions and concluded that youth with long-term physical disease had worse social functioning than healthy peers [20, 21]. The effects were larger for diseases associated with greater impairments in functioning (e.g. cerebral palsy, spina bifida) than diseases with lesser impairments in functioning (e.g. diabetes). Across disorders, the effects were larger when based on parent than child report.

The associations of peer relationships with health outcomes are more mixed. Even when supportive aspects have been identified, these can lead to negative outcomes, possibly as a result of being distracted from self-care and increased concerns about fitting in [19].

In the longer term, there is evidence that young people with long-term conditions [22] and with endocrine conditions [23–26] have lower rates of leaving the parental home or getting married.

Education and Vocation

Adolescence signifies a key stage in education and achieving vocational readiness. Having a long-term condition may disrupt education particularly if it developed during adolescence, resulting in significant periods of time out of education or missing lessons to attend routine clinic appointments or due to ill health. Similarly, educational attainment in this age group may result in a conflict of priorities with young people and their families prioritizing education over management of the condition during this time, resulting in, for example, missed appointments.

There is evidence that having a long-term condition may affect educational achievement and result in vocational impairments and loss of financial independence in adult life [22, 27]. Stronger differences were found for young people with neurological illnesses and sensory impairment than in individuals with other long-term conditions, particularly if the illness/disability is highly visible to others [22]. Two studies of the long-term impact of childhood-onset growth hormone deficiency found a lower level of education and income [24]

particularly in those with a past diagnosis of a brain tumour [23]. Differences from the general population in education and employment have also been identified in other childhood-onset endocrine conditions [25, 26].

Exploratory and Health-Related Behaviours

Exploratory and health-related behaviours are part of normal adolescent development with adolescents frequently being labelled as 'risk-taking'. Five of the 10 key risk factors for adult disease burden identified in the WHO Global Burden of Disease Study (tobacco, physical activity, overweight, unsafe sex and alcohol use) are problems usually initiated or heavily shaped in adolescence [24, 28]. These behaviours impact on adolescent health and track strongly into adult life [29], highlighting the importance of intervention in adolescence to prevent health burden.

Young people with long-term conditions exhibit these exploratory behaviours as frequently or more frequently than their health peers, including substance use, early sexual debut and violent or antisocial acts, and, compared with their health peers, were also more likely to report 3 or more than 4 risk behaviours [30]. The consequence of such exploratory behaviours in young people with long-term conditions may be greater than in their healthy peers and has been described as a 'double whammy' [31]. Such behaviours may also be associated with poor adherence to medication or reduced engagement with healthcare. Poor adherence itself may be one such behaviour as the young person explores the consequences and possible modes of future behaviour.

Developmentally Appropriate Healthcare

Due to the complexity of this period of life, health services must support the young person to engage with endocrine care; appropriate care in this age group is now called 'developmentally appropriate healthcare'. A recent review of the literature and a qualitative survey have identified the following themes that are required to deliver such care [32, 33]:

i) Biopsychosocial development and holistic care.
ii) Acknowledgement of young people as a distinct group.
iii) Adjustment of care as the young person develops.
iv) Empowerment of the young person by embedding health education and health promotion.
v) Interdisciplinary and inter-organizational work.

Designing Services to Meet Young People's Needs

The most effective way of designing a service to meet young people's needs is to involve them. Research has identified differences between the views of young people and the adults close to them, suggesting that adults cannot be used as proxies. Young people also benefit from such activities as it promotes citizenship and social inclusion, important for the health of the community. Moreover, it offers strategies for enhancing participation and will develop self-esteem and personal development. Guidance is available on how to involve young people in a meaningful way [34].

In a national survey of over 200 young people attending endocrine clinics in the UK, the greatest gaps in care were identified in aspects of the environment, followed by process and then by healthcare provider [35]. From an environmental perspective, adolescents suggested that not wanting time wasted in clinics and having an age-appropriate physical environment were aspects of care most in need of improvement. For process, they wanted more information to be provided about other people and organizations who can support them and other people to be informed about how their condition affects them. For healthcare providers, they wanted staff to whom they can talk about sensitive or difficult issues (even when they have nothing to do with their condition) and staff who understood the realities of being a young person.

In a systematic review of the literature addressing youth-friendly healthcare, Ambresin et al. [36] reported eight domains that stood out as central to young people's positive experience of care. These were as follows:

1) **Accessibility of healthcare**

This is key for young person friendliness and is potentially more of an issue in low-income countries. Accessibility could be related to either location or affordability. Accessibility also includes the ease with which the young person can contact the team for advice or information.

2) **Staff attitude**

A healthcare provider who could be described as young-person friendly should have accurate knowledge, be able to provide holistic care and have the following attributes: respectful, supportive, honest, trustworthy and friendly. The key attribute was respect. Friendly was described as being interested in non-medical aspects of their lives. Trust was more likely to develop if there was continuity of care and was associated with being able to discuss sensitive issues, feeling safe with their healthcare provider and being able to tell them anything. The attributes required also apply to non-medical staff such as receptionists.

3) **Communication**

The main aspects of communication emphasized by young people were the clarity and amount of information provided. A healthcare provider's listening skill was highlighted as an integral part of a good clinic visit.

Young people preferred a direct communication style without lecturing.

4) **Medical competence**

For young people attending endocrine clinics, this could translate to procedures such as venepuncture or injection technique. For other young people, pain management has been reported as an important aspect of good quality care.

5) **Guideline-driven care**

Young people desired holistic care, defined as relating not only to their condition but also to a developmental level, life events and personal aspirations. Transition was an issue in hospital care for young people with long-term conditions. The need for autonomy or promotion of autonomy was also identified. Routine discussion about confidentiality (and its limitations) was also key in a range of settings, especially in the context of psychosocial assessment.

6) **Age-appropriate environment**

Flexibility is needed in timing and type of appointments to minimize disruption to education. There is a growing literature on the use of video consultations in young people. More informal settings were identified. Other indicators included separate physical space for young people, up-to-date appropriate information in different formats available in the waiting room, television or games. Cleanliness was also raised as an issue. Long waiting times were interpreted by the young person as a lack of respect, as was privacy, for example, around weighing in clinic.

7) **Involvement in healthcare**

Young people stressed their need to be involved in their healthcare. This indicator was directly associated with a good understanding of their medical condition and treatment. Another opportunity to involve young people and enhance understanding is for clinic letters to be sent or copied to the young person rather than to their parents.

8) **Health outcomes**

Health outcomes in young people with endocrine conditions could potentially equate to quality of life and reduced admissions in the short term, to fertility and pregnancy in the medium term and to reduced morbidity and mortality in the long term.

Training Healthcare Providers

In the UK survey of young people and their parents attending endocrine clinics, healthcare provider characteristics were identified as the most important part of quality care [35]; however lack of staff training in adolescent health and/or transitional care has been reported as a major barrier for service delivery [37, 38]. In a UK survey of pediatric and adult trainees, training in adolescent and young adult health and transition was rated as minimal or non-existent by 65% of trainees, more marked in endocrinology (76%) than in diabetes (42%) [39].

There is evidence that training in adolescent health is beneficial. Sustainable improvements in knowledge, skill and self-perceived competence were reported in a randomized controlled trial [40] and screening for risk behaviours improved with training [41]. Training in adolescent health and transitional care could highlight aspects of healthcare such as triadic consultations with the physician, parents and the young person, cross-boundary and multidisciplinary working, ethics of consent and confidentiality or holistic care. There are online resources to support teachers <http://www.euteach.com> and online transition modules that can be accessed individually or used in small group work <http://www.e-lfh.org.uk>.

Clinic Consultations

The clinic consultation is the main or only intervention available to the endocrinologist to work effectively with young people and their parents. Good communication is central but can be challenging for the healthcare professional, particularly navigating the changing role of parents in a young person's care. Effective communication can be learned and improve health outcomes. Key issues when communicating with young people include the following [42]:

- The young person should be central in the communication, with discussions primarily focused on him/her.
- The young person should be offered time alone with the clinician.
- Conditional confidentiality should be discussed and does not reduce rates of disclosure.
- Ambivalence is normal and techniques such as motivational interviewing techniques can be learned to help the young person resolve ambivalence and change behaviour [43].
- A psychosocial screen is a key part of the adolescent consultation and should include resilience factors as well as risk – HEEADSSS is one such psychosocial screening tool [44, 45] (Table 19.2).
- Health promotion and education is another key part of the adolescent consultation and brief intervention strategies [46] are potentially useful, for example, the 5As of brief intervention: ask, assess, advise, assist and arrange.

The importance of the act of asking the right questions should not be underestimated, even when the young person is reluctant to answer. Brown et al. [47] reported that if consultations included discussion of a sensitive health topic, young people were more likely to have a positive

Table 19.2 HEEADSSS assessment for young people in endocrine clinics.

Theme	Relevance	Opportunity
Home	Identifying whether a young person feels happy and safe at home and has a supportive family who they can communicate with is a protective factor	Identifying safeguarding issues
Education and employment	Young people with endocrine conditions may have lower educational and vocational attainment for any number of intrinsic and extrinsic reasons	Assessing and addressing the impact of having an endocrine condition on education, training and employment
	Identifying whether a young person is engaged with education or training and has a plan for employment when they leave is a protective factor	Assessing and addressing vocational readiness including aspiration of the young person; educational achievement; prior work experience; knowledge of resources, responsibilities and rights; psychological aspects including self-esteem; expectations of the young person, their family, their teachers
		Addressing information needs of school, college, university, employment agencies and work to support the young person
		Discussing with the young person about disclosure to appropriate people about their condition to support education and vocation
		Offering neuropsychology for some young people, such as those treated for brain tumours, to guide them towards their strengths
Exercise and eating	Young people with endocrine conditions are at risk of having or developing obesity that is associated with low self-esteem and potentially longer-term consequences such as diabetes, cardiovascular disease, reduced fertility and sleep apnoea	Employing the 5As may assist in young people becoming more active and eating more healthily
	Young people who are childhood cancer survivors and have had treatment with the chemotherapy agent anthracyclines need advice about exercise such as weight lifting	
Activities (including peers)	Young people with an endocrine condition may have a disrupted peer network due to intrinsic or extrinsic factors	Enquiring about teasing and bullying may identify an area of risk and a young person in need of support
	Identifying young people with a supportive peer network and involvement in group activities is a protective factor	Exploring potential for peer interaction through patient support groups and youth groups or with the assistance of a youth worker
		Enquiring about the use of public transport and driving to support independence
Drugs (smoking, alcohol, other substances)	Young people with endocrine conditions are as likely as their healthy peers to explore the use of drugs	Enquiring whether family or peers smoke, drink alcohol or use drugs is a recognized risk even if the young person denies use
	Some young people with endocrine conditions are at risk of cardiovascular and/or respiratory disease, in particular childhood cancer survivors	Employing the 5As may assist in young people changing their drug use
	Young people on hydrocortisone or desmopressin who drink alcohol may increase their risk of adrenal crisis or hyponatraemia, respectively	Educating young people on hydrocortisone or desmopressin about keeping themselves safe when drinking alcohol
Sexuality	Young people with endocrine conditions are as likely as their healthy peers to become sexually active in adolescence and be at risk of unwanted pregnancy or sexually transmitted infections	Educating young people about sexual health and contraception and signposting to relevant services
	Young people with endocrine conditions may have questions about sexuality, ability to have sex and fertility	Discussing concerns about ability to have sex, in particular young people with disorders of sex development, and referring for further assessment if appropriate
	Young people with PCOS on anti-androgens should be advised about avoiding pregnancy	Discussing implications of their condition on fertility and referring for further assessment if appropriate
	Young people with endocrine conditions caused by genetic disorders or associated with cardiac concerns should be advised about prepregnancy counselling	Educating young people about the importance of prepregnancy genetic counselling and cardiac assessment and avoiding pregnancy in those on anti-androgens

Table 19.2 (Continued)

Theme	Relevance	Opportunity
Suicide, depression and self-image	Mental health issues are common in adolescence Protective factors include having an adult to talk to when stressed, a peer support network and coping skills, as well as positive self-esteem and image	Asking them to rate their general happiness to encourage discussion Asking about self-harm and suicide Referring to appropriate services if required
Sleep	Sleep problems in adolescence are common and can be a sign of anxiety and depression	Enquiring about sleep and discussing sleep hygiene
Safety	Safety about driving alone or with friends Safety about Internet use Safety about hydrocortisone and desmopressin in general and when away from home	Enquiring about safety with regard to driving and Internet use Educating about medic alert jewellery, sick day rules, emergency hydrocortisone injection and keeping safe with diabetes insipidus Discussing with the young person about disclosure to appropriate people (friends, colleagues) about their condition and offering to provide them with education Empowering them to insist on optimal care in healthcare settings

perception of the provider, have their worries eased, be allowed to make decisions and report taking responsibility for treatment.

The clinic consultation should be adapted to meet the needs of young people with learning disability. Although the developmental milestones are likely to be different, the young person should be encouraged to reach their full potential in the consultation including, if reasonable, lone consulting.

Addressing Adherence

Adherence to a treatment regimen requires appropriate cognitive capacities and personal organization as well as a personal belief that the treatment is required and beneficial [48]. Adherence is maximized when the professional–patient partnership decides management strategies in the light of the health beliefs and personal goals of the patient. In any discussion regarding adherence, it is important to reflect on what young people with endocrine conditions face day to day. They often are on lifelong hormone replacement and the benefits may not be immediately apparent to them and they may see medication as a potential barrier to partaking of leisure time, spontaneity and peer interactions. Non-adherent behaviour may be the only control mechanism open to the young person and be a simple wish to be heard and to take an active role in the decision-making process.

Addressing adherence in the clinic setting should never be about identifying 'poor' adherence. The most important strategy is to decriminalize non-adherence and give the young person opportunity to disclose difficulties that they are experiencing. 'When was the last time you forgot to take ….?' is a much more effective question than 'Do you remember to take …. every day?'. Adherence is multifactorial and assessed as in terms of health beliefs, previous experiences (first- or second hand), disease duration and maturity.

One of the common misunderstandings in dealing with non-adherence is that explanation about the rationale of therapy will suffice. For example, explaining that the young person may become seriously unwell if they do not take their hydrocortisone when this has not happened to date is not going to improve adherence. There is evidence that education alone is insufficient to improve compliance, and it is likely that a behavioural component is likely to be required [49].

Involving Parents

One of the foundations of care during adolescence and particularly during transition is that it is inclusive of the family. Parental issues have been reported to be significant during transition including some reports that transition is more difficult for parents than for the young people themselves. Parents found transfer 'easy' if they felt ready and perceived that the coordination between the teams was good [50]. A systematic review of parental involvement in transition concluded that parents can be key facilitators of their child's healthcare transition, supporting them to become experts in their own condition and care, but, to do so, they require clarification on their role and support from service providers [51]. A shared leadership approach should be encouraged with the parent moving from providing all the care in early childhood

to managing it with the young person participating during late childhood and, as their son/daughter becomes competent, for the parent to take the role of supervisor as the young person takes over the management, eventually becoming their own supervisor with the parental role evolving into that of consultant [52]. Studies are underway to identify effective ways of supporting parents in this changing role.

Transition and Transfer to Adult Services

All young people require developmentally appropriate healthcare and some young people require transition and transfer to adult services. The definition of transition is 'a multi-faceted, active process that attends to the medical, psychosocial and educational/vocational needs of adolescents as they move from child- to adult-centred care' [53]. The aims of transition are to:

- Provide high quality, coordinated, uninterrupted healthcare that is patient centred, age and developmentally appropriate, culturally competent, flexible, responsive and comprehensive with respect to all persons involved.
- Promote skills in communication, decision-making, assertiveness and self-care, self-determination and self-advocacy.
- Enhance the young person's sense of control and interdependence.
- Provide support for the parent(s)/guardian(s) of the young person during this process.
- Maximize lifelong functioning and potential.

Transition is a long process, starting in early adolescence and finishing in young adulthood; transfer is one event within it. Timing of events within transition and transfer depends on many variables and must be individualized to the young person concerned (Box 19.1).

Box 19.1 Factors to consider in the timing of transition and transfer.

- Chronological age
- Physical maturity
- Cognitive maturity
- Current medical status
- Adherence to therapy
- Independence in healthcare
- Preparation
- Readiness of the young person
- Readiness of the parent/caregiver
- Availability of an adult endocrinologist with expertise
- Readiness of adult endocrinology service

Evidence Base for Transition and Transfer

There is evidence that transition and transfer could be improved for some young people with endocrine conditions [54, 55] and other long-term conditions, as some are lost from follow-up, are dissatisfied with care and suffer a deterioration in health [56]. The evidence base for improving transition and transfer in endocrinology is limited but the evidence base across long-term conditions, including diabetes, continues to evolve and is applicable [57–59]. Due to the minimal evidence base, a Delphi study [60] has allowed the synthesis of the evidence and expert opinion and key elements are recorded in Box 19.2.

Monitoring Transition and Transfer

With the lack of evidence of the best way to transition and transfer young people, monitoring of outcomes is key [56]. The same Delphi study identified the following indicators of successful transition [60] recorded in Box 19.3.

Young People with Endocrine Conditions

There are broadly three groups of young people attending endocrine clinics:

1) Young people presenting in adolescence due to the variations in the hormonal milieu around this time with concerns about stature and progress through puberty, menstrual irregularity and hyperandrogenism in females and gynaecomastia in males.
2) Young people with either a congenital or acquired condition from childhood who may either have been diagnosed earlier and have grown up receiving pediatric endocrine care to maximize growth and optimize the timing of puberty and screen other aspects of health or may present for the first time late in adolescence and have missed out on timely pediatric endocrine care.
3) Young people presenting with conditions more frequent in adult endocrine practice like autoimmune conditions most commonly affecting the thyroid and, although still rare in adolescence, pituitary tumours, most frequently prolactinomas.

The approach to a young person with an endocrine condition depends on the condition and the timing of its presentation and includes the following aims:

- To ensure the young person who presented earlier in childhood has all the information previously shared

Box 19.2 Key elements of healthcare during transition.

1) Assuring a good coordination (such as timing of transfer, communication, follow-up, remaining available as a consultant, etc.) between pediatric and adult professionals
2) Starting planning transition at an early age (and at least 1 year before the transfer boundary)
3) Discussing with patient and family about self-management
4) Including young person's views and preferences to the planning of transition
5) If developmentally appropriate, seeing the adolescent alone at least for part of the consultation
6) Identifying an adult provider willing to take on the young patient before transfer
7) Tailoring the transition plan to the needs of the patient and family
8) Identifying someone within the team who will play the role of transition manager or coordinator
9) Providing a written health summary and biopsychosocial profile summary to patient and adult care provider before transfer
10) Having a written transition protocol/plan that is available to patients, parents and providers
11) Making sure that at least one appointment with adult provider after transfer is fixed in anticipation
12) Pediatric and adult clinics making sure they have sufficient knowledge and skills in adolescent health
13) Parents/legal representatives should be included in the process of transition
14) Informing primary care provider (pediatrician, GP, family doctor, nurse practitioner/advanced practice nurse) of the transition process
15) Putting in place mechanisms/resources to contact patients lost to follow-up
16) Discussing with patient and family about differences between pediatric and adult care
17) Discussing with patient about risk behaviours and their influence on health

Box 19.3 Key indicators of a successful transition.

1) Patient not lost to follow-up
2) Attending scheduled visits in adult care (no missed consultations unless previously cancelled and rescheduled)
3) Patient building a trusting relationship with adult provider
4) Continuing attention for self-management
5) Patient's first visit in adult care no later than 3–6 months after transfer
6) Number of ER visits for regular care in the past year (avoidable if routine medical care had been occurring)
7) Patient and family satisfaction with transfer of care
8) Maintain/improvement of standard for disease control evaluation (such as glycosylated haemoglobin for diabetes, for example)

with their parents about their diagnosis, previous investigations and management.

- To address the priorities of the young person presenting later in adolescence, particularly in respect of delayed puberty and short stature.
- To discuss management with the young person regarding cardiovascular, bone and reproductive health, as well as quality of life.
- To raise awareness of maintaining safety for those on hydrocortisone and desmopressin and recognizing ill health and what to do and when to seek help.
- To identify level of endocrine care required in adult life and whether care can be provided by the family doctor, for example, in congenital hypothyroidism, or whether transfer to adult services is required.

In addition, more generic approaches are also important:

- To encourage increasing independence in healthcare including organizing medication and prescriptions, arranging appointments at times suitable to them, coming in for part or all the consultation independently of their parents and contacting the hospital in between appointments to seek information and advice.
- To address barriers to engagement with healthcare including adherence.
- To discuss useful resources to get health information and support including patient support group.
- To address concerns about venepuncture or injections, particularly around transfer to adult services.
- To highlight benefits and ways of disclosure about condition to friends, teachers and employers.
- Through psychosocial screening (see Table 19.2) to address broader issues such as psychological, social and educational/vocational issues and health behaviours by identifying risk and protective factors.

Involvement of the multidisciplinary team is important at this time; pediatric and adult endocrinologists and endocrine nurses need to liaise with each other and some involvement of a clinical geneticist, psychologist, gynaecologist and/or urologist and fertility specialist would be essential.

Endocrine Care in Adolescence

There are aspects of endocrine care that are unique to the period of adolescence. Some are highlighted here.

Symptoms and Signs of PCOS in Adolescence

Polycystic ovary syndrome (PCOS) is the most common female endocrine disorder, with many women initially presenting during adolescence. The pathogenesis of PCOS is hypothesized to be based on interactions between genetic and environmental factors.

PCOS in adult women is diagnosed when two of three criteria, namely, chronic anovulation, hyperandrogenism or hyperandrogenaemia and polycystic ovaries on ultrasound, are present [61, 62]. In the adolescent age group, these criteria should be employed with caution. Acne is common during the adolescent years, whereas hirsutism associated with PCOS typically develops over time. Normal ranges for androgen measurements have not been established in adolescents. Irregular menses are common due to the immaturity of the hypothalamic–pituitary–ovarian axis in the 2–3 years following menarche. In adolescent girls, large, multicystic ovaries are a common finding.

Consequently, there is no overall agreement as to how to diagnose PCOS in adolescence. Some investigators suggest that all three elements of the Rotterdam criteria should be present in adolescents to make the diagnosis [63], whereas others have suggested that hyperandrogenism or hyperandrogenaemia with persistent oligomenorrhoea should be present to make the diagnosis and anovulatory symptoms and ultrasound appearance should not be part of the diagnostic criteria in patients of this age group [61]. It has also been suggested that the diagnosis should not be made until the age of 18 [64].

A collaboration between international pediatric and adolescent specialty societies reviewed the available literature [65]. The authors conclude that uterine bleeding at intervals more frequent than 19 days or less frequent than 90 days is abnormal even in the first post-menarcheal year. In the absence of clinical evidence of an endocrine disorder, persistent abnormal menstrual bleeding for 1 year carries an ~50% risk of ongoing menstrual irregularity, and approximately half of the ongoing cases will have PCOS. However, if clinical evidence of PCOS is present, such as hirsutism, the risk of ongoing hyperandrogenic menstrual abnormality is high. They have therefore proposed that PCOS be diagnosed in adolescence in the setting of an otherwise unexplained combination of [66]:

1) Abnormal uterine bleeding (AUB) pattern that is persistent for 1–2 years:
   a) Primary amenorrhoea – Lack of menarche by 15 years of age or by 3 years after the onset of breast development.
   b) Secondary amenorrhoea – Over 6 months without a menstrual period after initially menstruating.
   c) Oligomenorrhoea (infrequent AUB):
      i) Post-menarcheal year 1: Average cycle length 90 days (4 periods/year).
      ii) Post-menarcheal year 2: Average cycle length 60 days (6 periods/year).
      iii) Post-menarcheal years 3–5: Average cycle length 45 days (8 periods/year).
      iv) Post-menarcheal years >6: Cycle length 38–40 days (9 periods/year).
   d) Excessive anovulatory AUB menstrual bleeding that occurs more frequently than every 21 days (19 days in year 1) or is excessive (lasts 7 days or soaks 1 pad or tampon every 1–2 hours).
2) Evidence of hyperandrogenism:
   a) Persistent testosterone elevation above adult norms in a reliable reference laboratory is the best evidence.
   b) Moderate to severe hirsutism is clinical evidence of hyperandrogenism.
   c) Moderate to severe inflammatory acne vulgaris unresponsive to topical treatment is an indication to test for hyperandrogenaemia.

There is limited understanding of the psychosocial challenges that patients with PCOS face. Reviews of the literature showed that PCOS has a negative influence on HRQoL in adolescents [67] and an increased risk of moderate and severe depressive and anxiety symptoms in adult women [68]. Body weight issues and body mass index (BMI) appeared to have the strongest effect on HRQoL in adolescents with some studies reporting a normalization of HRQoL scores after adjustment for BMI [67]; however in adults the symptoms are weakly associated with age, BMI, elevated testosterone, hirsutism and insulin resistance [68].

Consequently although diagnosis during this period is challenging, adolescents who exhibit symptoms of PCOS should be counselled and managed to address psychosocial issues and prevent the long-term complications of the syndrome, especially subfertility and cardiovascular disease. The recommendation is therefore that individual manifestations of PCOS should be treated following evaluation.

Healthy-living discussions remain the cornerstone of management, particularly for obese or overweight adolescents but also for those with a healthy weight about maintaining it. Otherwise treatment is symptom directed. Evidence is low quality for management in

adolescence [69, 70]. Hair removal with laser and other measures should be explored. A combined oral contraceptive pill is ideal to improve both the menstrual abnormality and hyperandrogenism, unless there are contraindications when cyclic progestin with or without anti-androgens should be considered. If anti-androgens are being considered, discussion about contraception is essential. Metformin may be suitable for those with evidence of insulin resistance or impaired glucose tolerance to improve menstrual irregularity.

Pubertal Gynaecomastia

50–60% of adolescent males have asymptomatic gynaecomastia [71, 72]. An increase in oestradiol concentration, lagging free testosterone production and increased tissue sensitivity to normal male concentrations of oestrogen are possible causes [73]; a longitudinal study also identified elevated IGF-1 as a possible contributor in boys [74].

Pubertal gynaecomastia should resolve within 6 months to 2 years after onset. If symptoms persist after 2 years or past 17 years of age, further evaluation is indicated for non-physiological causes.

In a series of young adult patients with gynaecomastia aged 19–29 years of age, a cause other than idiopathic was identified in 42%: hypogonadism (25%), hyperprolactinaemia (9%), chronic liver disease (4%) and drug-induced gynaecomastia (4%) [75]. The potential of a testicular or adrenal tumour should also be considered.

Although the condition in most adolescents is physiological, it can have significant psychological and social consequences that include but are not limited to depression, anxiety, disordered eating, body dissatisfaction and reduced self-esteem [76, 77].

In those in whom resolution of gynaecomastia is still a possibility, reassurance may be sufficient, but management options should be discussed even in those patients due to psychological factors. There is evidence that tamoxifen is safe and may be effective for the treatment of pubertal gynaecomastia but randomized controlled studies are lacking [78]. Surgery to improve cosmetic appearance may be warranted [79].

Ongoing Management of Congenital and Acquired Conditions Diagnosed in Childhood

The focus of care in late adolescence looking forward to adult health is related to cardiovascular, bone and reproductive health from the perspective of monitoring and optimizing hormone replacement. In adolescents after the achievement of adult height, there are different approaches in the management of growth hormone deficiency (GHD), oestrogen deficiency, congenital adrenal hyperplasia and glucocorticoid replacement.

Childhood Growth Hormone Deficiency in Adolescence

In 2003 there was a consensus meeting focussing on the management of patients treated with rhGH for GHD during childhood [80]. Based on the available evidence at that time, it was recommended that ongoing rhGH should be offered to all adolescents at the end of growth who remain GHD. The rationale for continuing included optimizing body composition and bone mass.

Most studies show adverse changes in body composition in those with persistent GHD who discontinued rhGH for 2 years, with decreasing lean mass (8%) and increased fat mass (10–17%) [81–84]. Another study using peripheral quantitative computed tomography (pQCT) identified adverse changes after 6 months [85]. Favourable improvements in body composition were observed on restarting or continuing rhGH, with an increase in lean mass (14%) and reduction in fat mass (7%) over 2 years [86, 87]. Only one study showed no difference [88]. Data on the effects of GHD or rhGH on muscle strength is inconclusive [89].

The studies exploring bone yield more inconsistent results. Of the studies looking at the impact of discontinuation, one found no change in bone parameters at 2 years [90], whereas another showed a lower femoral neck BMD after 6–12 months off rhGH [91]; a study using pQCT showed a reduction in cortical thickness after 6 months [85]. The studies looking at continuation of rhGH demonstrate increases in TB-BMC and LS-BMD at 1 [92] or 2 years [86, 93, 94], similar to what would be expected in the normal population, and after 2 years a significant increase in cortical thickness has been observed [95], although others have shown no benefit [81, 88]. Importantly, the fracture data do not support the hypothesis that patients with childhood-onset GHD are at increased risk [96]. Assessment of bone quality, using more advanced non-invasive imaging tools, may provide a greater insight into the effects of GHD and rhGH on the bone [89].

Other areas studied include cardiovascular risk and lipid metabolism and quality of life [89]. While a more adverse lipid profile develops following discontinuation, the impact of continuing and restarting rhGH is less clear and there is no long-term evidence of adverse outcomes. With regard to quality of life, recognized as an area that potentially improves in adults with GHD, the use of different tools and a lack of an appropriate well-validated tool for this patient group have led to studies that have not consistently demonstrated benefit.

The variability in the effect of GHD and rhGH in these studies is in part due to the different populations, including the duration and severity of GHD and the presence of other pituitary hormone deficiencies and duration on and off rhGH.

Despite this limited evidence, retesting for GHD and discussion about restarting rhGH should persistent GHD be confirmed is recommended.

Retesting is essential as patients diagnosed as GHD in childhood will not necessarily be GHD in late adolescence and young adulthood, with apparent recovery in significant numbers of GHD patients. Different GH cut-offs are used to diagnose GHD at the time of retest. The apparent recovery has been attributed to long-term exposure to sex steroids potentially augmenting pituitary size and GH secretion, transient GHD and incorrect diagnosis due to issues with biochemical testing [97]. The underlying aetiology will likely determine the likelihood of persistent GHD: more than two-thirds of children diagnosed as GHD in childhood will be normal on retesting, particularly those with idiopathic, isolated or partial GHD [98, 99]. Only 61% of patients with childhood GHD and an ectopic posterior pituitary remained severely GHD on retesting using a GH cut-off of <5 mcg/L [100]. In childhood cancer survivors treated with cranial irradiation during childhood, 52% of the whole cohort were eligible for GH in adulthood, increasing to 64% in those with severe GHD (peak GH <3 mcg/L) in childhood [101].

This information allows retesting of GH status at end of growth to be stratified into those that do not need retesting (very high likelihood of persisting GHD) and those with a high and low likelihood of persistent GHD.

The first European Society for Paediatric Endocrinology (ESPE) consensus identified those with panhypopituitarism (defined as four or five hormone deficiencies) as not needing retesting [80] and the GH Research Society in association with ESPE and other societies suggested guidelines that extended the cohorts of patients that did not require retesting to those with known transcription factor or genetic mutation [102]. In these groups of patients, rhGH can be continued seamlessly if the young person agrees.

In the ESPE consensus [80], those with a high likelihood of having persistent GHD were defined as those with severe GHD in childhood due to organic hypothalamic–pituitary abnormalities (e.g. central nervous system [CNS] tumours affecting the hypothalamic–pituitary region, those who received high-dose cranial irradiation, those with structural pituitary abnormalities [e.g. pituitary stalk agenesis and ectopic posterior pituitary depending on position]). These require retesting a month after finishing rhGH for linear growth and they may initially require only measurement of IGF-1 concentration, progressing to provocative testing only if the IGF-1 is in the normal range. Patients with a low likelihood of persistent GHD include all other patients treated with rhGH for GHD during childhood, particularly those with idiopathic GHD and no organic pituitary abnormality, and these patients require both IGF-1 estimation and provocative testing.

A low IGF-1 alone is recommended to be taken as sufficient evidence of GHD without further provocative testing in those with a high likelihood of persistent GHD [80, 103]. Only one provocative test is recommended by all the consensus statements in those with a high likelihood when IGF-1 is within the normal range and in those with a low likelihood of persistent GHD [80, 102, 103], although the endocrine guidelines highlight that as idiopathic GHD in adults is very rare, more stringent criteria are suggested including the performance of two provocative tests, particularly if the IGF-1 is in the normal range [103].

The insulin tolerance test (ITT) has been validated for the diagnosis of persistent GHD in the transition period. The two consensus documents offer different GH cut-offs [80, 102]; the most recent suggests <6 μg/L although it comments that further evaluation is required [102]. This figure is based on the finding that a GH cut-off of 5.6 μg/L during an ITT had the highest accuracy for predicting persistent GHD with a sensitivity of 77%, specificity of 93% and correct classification of 87% of patients [104]. A similar study identified that a GH cut-off of 6.1 μg/L during an ITT had a sensitivity of 96% and specificity of 100% [105]. More recently, a study looking at cut-offs in children and adolescents suggested that the optimal cut-off during an ITT was 5.1 μg/L, that during an arginine stimulation test was 6.5 μg/L, and that for clonidine was 6.8 μg/L [106]. The other test with supporting evidence in the transition period is the GH-releasing hormone (GHRH)–arginine test and a higher cut-off compared with adults (19 μg/L) had an optimal sensitivity and specificity [107]. GHRH–arginine test results may be falsely normal in individuals with hypothalamic dysfunction. Although the ITT and GHRH–arginine test have been validated for the diagnosis of persistent GHD in the transition period, GHRH is not always available and the ITT is contraindicated in some individuals and not practical in many endocrine practices. The arginine test can be used but the response is dependent on BMI so the arginine test should be limited to non-obese adolescents. Glucagon stimulation testing is a promising alternative for provocative testing in adults but has not been tested in the transition period.

Some groups may require further re-evaluation, regardless of the results of initial testing, at the end of growth as they are at risk of evolving hypopituitarism, including GHD. This is particularly the case for those treated with radiotherapy and patients with ectopic posterior pituitaries who may develop GHD in late adolescence despite initial normal testing.

Further evaluation using adult criteria around the time of completion of growth (~25 years) can be considered

but is likely to be dependent on the severity of GHD and the local threshold for GH replacement in adult life. Those with low likelihood of persistent GHD as described above should be revaluated before a commitment is made to lifelong GH replacement. Re-evaluation at this time may also be justified for patients in the low likelihood group who had discordant tests (normal IGF-1; GHD on provocative testing OR low IGF-1; normal GH on provocative testing).

The optimal GH dose during the transition period is not clear. Studies used GH doses that varied from 12.5 to 25 μg/kg/day (weight based) to 200 μg/day (fixed dosing) [86, 87, 93, 108]. With respect to bone health and body composition, one study demonstrated no dose dependency [87, 93], whereas another found that a higher dose had a greater effect on spine BMD compared with a lower dose. Although similar effects were observed with LBM and FM at 6 months between the two doses, these were more likely to be sustained with the higher dose [86]. This study also demonstrated a greater improvement in LDL-C with the higher dose of rhGH [86]. Neither study demonstrated a dose effect in relation to quality of life [86, 108].

Some differences in dosing based on age have been recommended by the Endocrine Society Guidelines for Evaluation and Treatment of Adult Growth Hormone Deficiency; patients <30 years of age may benefit from initial doses of 0.4–0.5 mg daily (as opposed to the initial doses of 0.2–0.3 mg daily for patients aged 30–60 years) and those transitioning from pediatric to adult replacement may need even higher doses. Females receiving oral (but not transdermal) oestrogen may need higher doses than other patients. Doses should be titrated subsequently to normalize serum IGF-I concentrations for age and gender.

This evidence, or the lack thereof, for the benefits of GH replacement in late adolescence and young adulthood allows the endocrinologist to give a clear choice to young people with persistent GHD as to whether their preference is to continue GH replacement in the transition period with potential benefit or have a holiday from daily injections, which may be preferable for many reasons.

Oestrogen Replacement in Adolescents

Girls with gonadotropin deficiency or premature ovarian insufficiency (POI), including Turner syndrome and some childhood cancer survivors, require oestrogen replacement throughout life. There are choices for the young person to consider, whether they are presenting in adolescence with delayed puberty or have completed pubertal induction and are considering their preferred oestrogen replacement in adult life. Guidelines for POI [109] and Turner syndrome [110] detailing the rationale for the most appropriate approach to oestrogen replacement have been published.

Girls with delayed puberty require an individualized approach to ensure that the priorities for achieving maturity or maximizing remaining growth are met. A shortened protocol for pubertal induction is generally successful, although available studies are too small to identify the reasons for unsatisfactory pubertal outcomes such as breast hypoplasia [111]. No data on long-term outcomes such as peak bone mass are available.

After pubertal induction, optimizing oestrogen replacement is important because oestrogen deficiency, particularly in the setting of POI, is associated with reduced life expectancy largely due to cardiovascular disease [109]. Oestrogen deficiency is also associated with reduced bone mineral density (BMD) and an increased risk of fractures later in life [109, 110]. Despite lack of longitudinal outcome data, early initiation of oestrogen replacement is strongly recommended to control future risk of cardiovascular disease and optimize BMD; it should be continued at least until the average age of menopause. Lifestyle measures, such as regular weight-bearing exercise, avoiding smoking, maintaining a healthy body weight and adequate dietary calcium and vitamin D, are important.

Oestrogen prescribing for pubertal induction varies between countries and clinicians. After pubertal induction is complete, there is now a move towards offering young women with oestrogen deficiency physiological oestrogen replacement in the form of hormone replacement therapy (HRT) rather than the combined oral contraceptive pill, unless there are concerns about the possibility of spontaneous pregnancy. The reason for this move is based on HRT being more favourable for bone health and cardiovascular health with lower blood pressure, better renal function and less activation of the renin–angiotensin system [109].

Evidence for the optimal mode of oestrogen administration (oral or transdermal) is inconclusive. Transdermal oestradiol results in more physiological oestrogen concentrations and is preferred in young women with obesity, hypertension, migraines and increased risk of venous thromboembolism [109]. Cyclical progestogen is required to maintain the health of the endometrium. Young women and their parents, particularly their mothers, should be reassured that concerns about the increased risk of breast cancer with HRT do not apply to this age group [109].

Altered sexual function and genitourinary symptoms may still occur despite adequate oestrogen replacement [109]. These should be discussed with young people so that they are aware that this is a discussion that can be had at their appointment. If these issues do occur, options such as increasing oestrogen dose as well as lubricants

and local oestrogen can be considered. For some women with POI and reduced libido, the use of testosterone supplementation could be considered, although young women should be counselled about lack of data on long-term efficacy and safety, so that duration should potentially be restricted to 24 months.

Routinely annual evaluation should include smoking status, BMI and blood pressure; in some groups, such as those with Turner syndrome and some cohorts of survivors of childhood cancer and those with brain tumours and following bone marrow transplantation, this should also include a lipid profile and HbA1c. Assessment of bone mineral density (BMD) should be considered, particularly if other risk factors are present, and repeated at 5 years if evidence of osteoporosis has been found [109]. Appropriate adjustments to BMD should be made for short stature. A decrease in BMD should prompt review of oestrogen replacement and other potential factors.

Glucocorticoid Replacement in Adolescents with Congenital Adrenal Hyperplasia

Management of adolescents with congenital adrenal hyperplasia (CAH) not only presents challenges but also offers opportunities to involve young people in making decisions about their management [112]. Control can deteriorate in adolescence due to physiological changes during puberty [113] and reduced adherence to medical therapy. Once linear growth is complete, there is a shift in focus of treatment to prevention of long-term adverse outcomes such as subfertility secondary to testicular adrenal rest tumours or menstrual irregularities, increased cardiovascular risk and osteoporosis. These long-term adverse outcomes are in evidence during adolescence [114].

In adolescents with classical CAH, education about avoiding and recognizing signs and symptoms of adrenal crisis is key, as there is evidence that adrenal crises in CAH increase in frequency in young adulthood [115]. Although it is accepted that lifelong glucocorticoid therapy is required, the glucocorticoid regimen should be reassessed after completion of growth and puberty, particularly in those struggling to achieve optimal control. While hydrocortisone is almost exclusively used in paediatrics for the management of CAH, studies from the UK and USA demonstrate that two-thirds of adults are on long-acting glucocorticoid preparations such as dexamethasone or prednisolone [116, 117]. There are no randomized controlled trials comparing these regimens; however a recent UK study did identify that increased adiposity, insulin resistance and use of prednisolone or dexamethasone are associated with impaired QoL in adults with CAH [118]. While hydrocortisone may be the optimal glucocorticoid, in some the priority will be simplifying the glucocorticoid regimen and changing to a long-acting glucocorticoid once or twice daily as required. Long-acting hydrocortisone preparations are being trialled in patients with CAH [119]. Choice of long-acting glucocorticoid may depend on circumstances. In adolescent males with testicular adrenal rest tumours, short-term dexamethasone may potentially preserve or restore fertility [120]. In females who are heterosexually active without adequate contraception, dexamethasone should be avoided because the placenta does not inactivate it, unless she is planning pregnancy and has been assessed by a clinical geneticist as being at risk of having an affected child when the pros and cons of dexamethasone should be discussed. The aim is for the lowest dose of glucocorticoid to normalize androstenedione concentrations but maintain 17OHP concentrations above the normal range. Long-acting glucocorticoid medications have reduced mineralocorticoid effects but monitoring plasma renin activity and blood pressure is always indicated. Fludrocortisone doses should be adjusted to maintain plasma renin activity within the normal range.

Patients with non-classic CAH represent a group less at risk of adrenal crisis and consequently may not need glucocorticoid therapy continuously lifelong. Guidelines suggest that patients should be given the option for discontinuing glucocorticoids [121]. Some authors have recommended weaning boys with non-classical CAH off hydrocortisone from mid-puberty and girls 2–3 years after achieving menarche [112]. In patients diagnosed in late adolescence, glucocorticoid therapy might be indicated only at specific times. In females, glucocorticoids may be required to regularize periods to enhance fertility and reduce rate of miscarriage [122]; other options such as the use of the combined oral contraceptive pill and anti-androgens are available for other symptoms in those not seeking fertility [123]. Assessment of adrenal function is important as around a third will have subnormal short Synacthen tests and, despite a lack of evidence in relation to the occurrence of adrenal crisis, the advice to cover sickness with hydrocortisone should be emphasized [124, 125]. Although data are lacking for long-term outcomes in non-classical CAH, follow-up into adulthood is advised, particularly in females who may require management of hyperandrogenaemia or assistance with fertility.

Conclusion

Addressing the health needs of adolescents and young adults with endocrine conditions requires developmentally appropriate healthcare and, for some, effective and safe transition and transfer to adult endocrine services. More evidence is required to inform how to deliver

effective care to this age group. Paediatric and adult endocrinologists need to recognize the implications of having an endocrine condition on psychological and social development. They need the knowledge about common presentations of endocrine conditions and management during adolescence to allow appropriate counselling of patients about their choices and involve them in their care.

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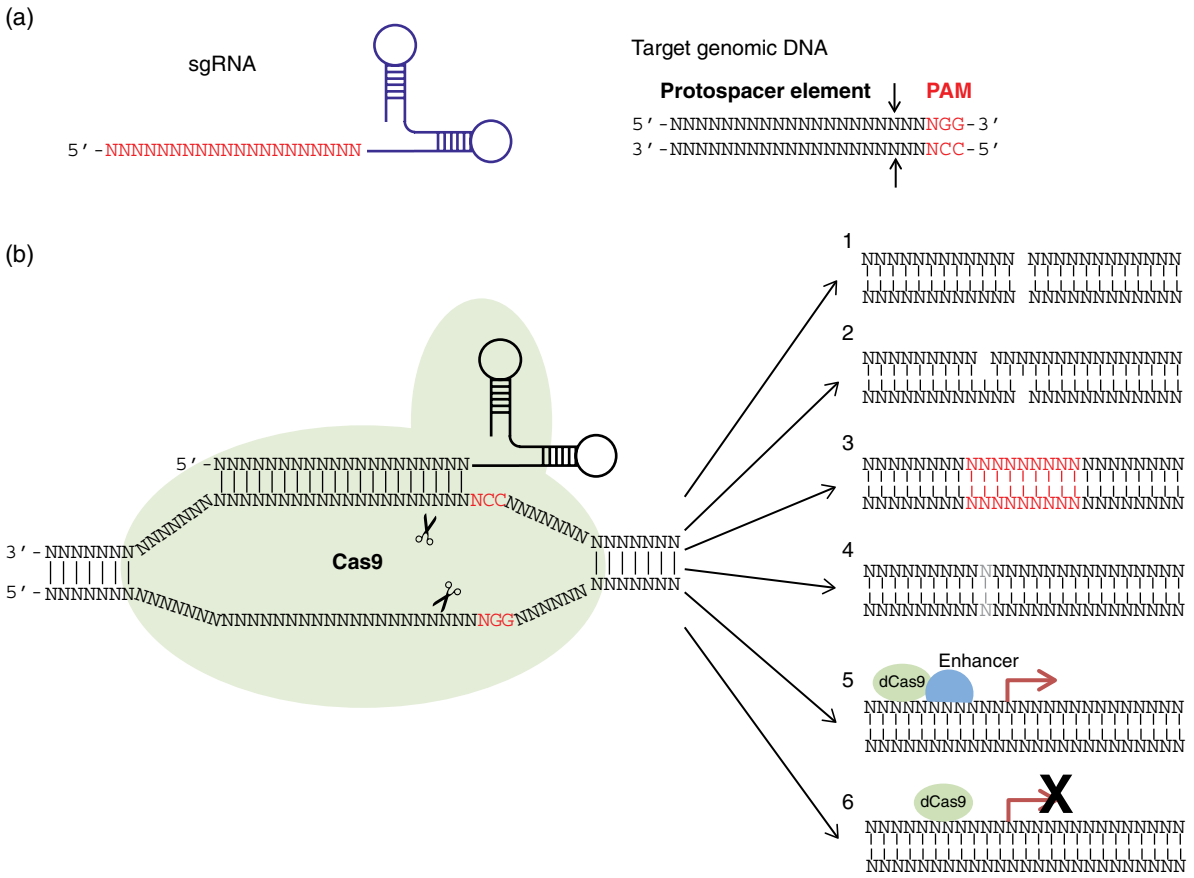

Figure 1.6 Overview of the main CRISPR/Cas9 system and experimental approaches. (a) Left, schematic representation of a single guide RNA, which is a synthetic RNA molecule resulting from the fusion of crRNA (red) and the scaffold tracrRNA (blue). Right, the target DNA sequence. This consists of a protospacer element of 20 nucleotides (black) immediately upstream of the protospacer adjacent motif (PAM), NGG (in red). The arrows indicate the position of the double-stranded break created by the Cas9 nuclease. (b) Left, schematic representation of the CRISPR/Cas9 sgRNA system at the target DNA. Right, different types of genome manipulation are represented. (1) Double strand break. (2) Single-stranded breaks using a modified Cas9 termed Cas9 nickase. Two adjacent sgRNAs targeting different strands will lead to nicks in the DNA and the possibility to create an insertion or deletion. This increases target specificity. (3) It is possible to tag protein encoding genes with either a fluorescent protein or a protein that acts as a selectable marker for the selection of successful knock-in events, for example. (4) Introduction of a single point mutation. (5) A catalytically inactive Cas9 (dCas9) fused to a transcription activator peptide can induce or increase transcription of a gene with cutting the DNA. (6) CRISPR/dCas9 can sterically repress transcription by blocking transcriptional initiation or elongation. Fusing a repressor domain to dCas9 allows transcription to be further repressed by inducing heterochromatinization.

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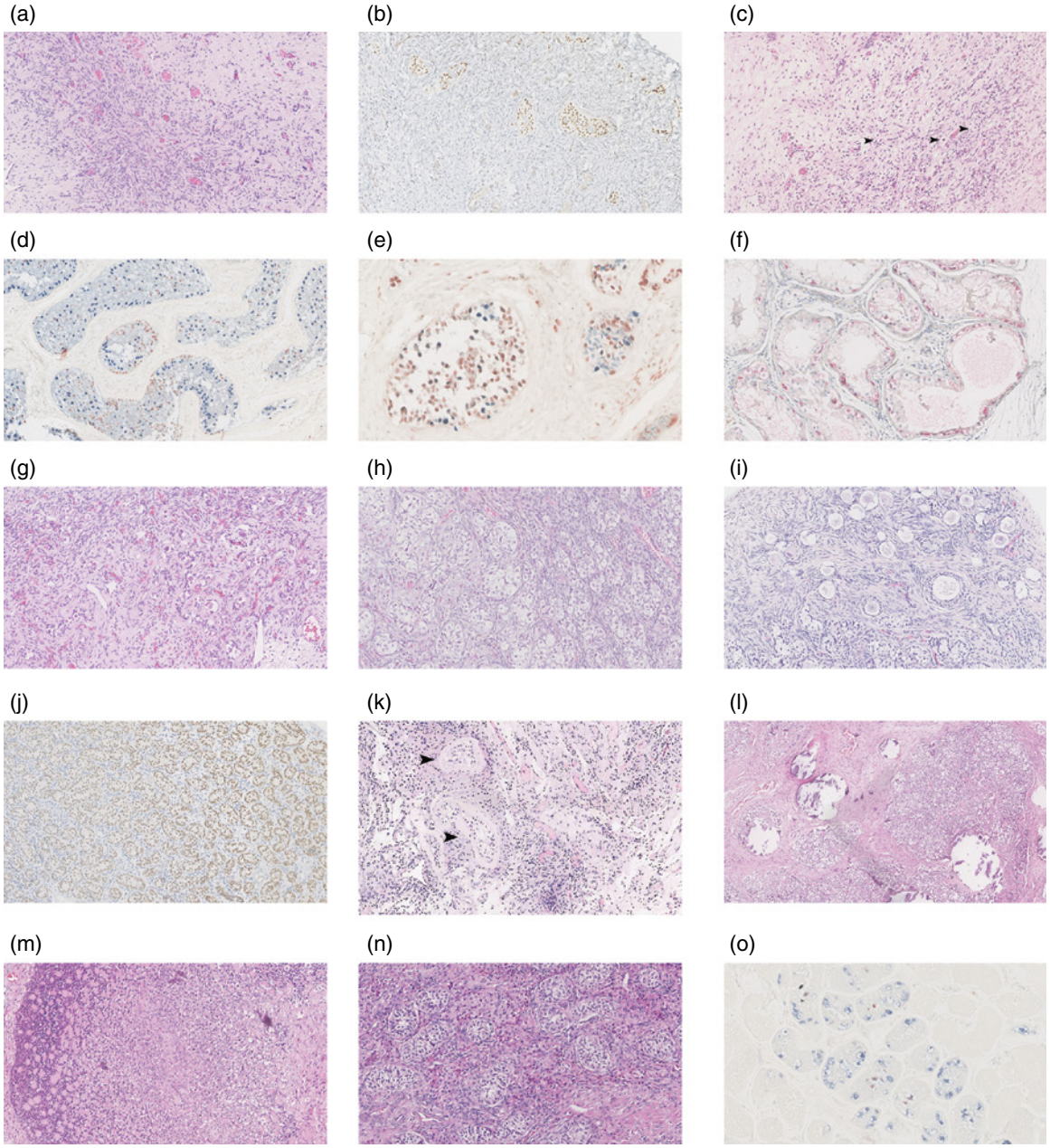

Figure 4.4 Examples of gonadal differentiation patterns, illustrating the variability of possible gonadal outcomes and co-presence of characteristics of both the male and female pathways in many dysgenetic gonads, as opposed to gonads from individuals with hormonal defects. (a) Streak gonad in an individual with 45,X/46,XY mosaicism. HE staining, 200×. (b) Streak gonad in another individual with 45,X/46,XY. Some cells have differentiated as Sertoli cells (SOX9 positive, brown staining) and form tubular aggregates. SOX9 staining, 200×. (c) Undifferentiated gonadal tissue in a 46,XY individual with *WWOX* mutation. Germ cells (arrowheads) form aggregates with pre-Sertoli/granulosa cells in a background of fibrous stroma. Testis tubules or ovarian follicles cannot be readily recognized. HE, 200×. (d) Partial gonadal dysgenesis in a 46,XY individual of unknown aetiology. Testis tubules contain both cells with Sertoli (blue) and granulosa (red) cell differentiation. SOX9–FOXl2 double staining, 200×. (e) Gonadoblastoma in the same individual. Supporting cells in a gonadoblastoma context are mostly (pre-)granulosa cells. SOX9–FOXl2 double staining, 400×. (f) Carcinoma *in situ* (CIS) in the same individual. Premalignant germ cells (TSPY positive, red staining) are stuck to the thickened basal membrane; the supportive Sertoli cells are shifted away from the basal membrane towards the internal side of the germ cell layer. Note that the supporting cells in a CIS context are Sertoli cells as opposed to gonadoblastoma where the supportive cell line consists of (mainly) granulosa cells. (g) Area of undifferentiated gonadal tissue in an individual with 45,X/46,XY. Many germ cells can be recognized, sometimes isolated in fibrous stroma, and sometimes arranged in clusters with pre-Sertoli/pre-granulosa cells. HE staining, 200×. (h) Gradual transition from testis (left) to a more undifferentiated pattern (right) in an individual with 45,X/46,XY. HE, 200×. (i) Ovotesticular DSD in a 46,XX SRY-negative individual. Ovarian differentiation in the upper part, testis in the lower part. HE, 200×. (j) Testicular DSD in a 46,XX SRY-negative individual. In spite of the absence of SRY, there is clear SOX9 expression (brown staining) in small (infantile) Sertoli cell-only tubules. SOX9 staining, 200×. (k) Testis atrophy with loss of testicular architecture in an older (40 years) individual with 46,XX testicular DSD. Scarce hyalinized atrophic Sertoli cell-only tubules (arrowheads) in a background of fibrous stroma. HE, 200×. (l) Large gonadoblastoma in an individual with 45,X/46,XY. HE, 100×. (m) Same gonad, dysgerminoma component. HE, 200×. (n) Typical testis differentiation but with marked Leydig cell hyperplasia in a 46,XY adolescent with CAIS. The intertubular space is packed with Leydig cells. HE, 200×. (o) Testis of a 3-year-old CAIS individual showing maturation delay of germ cells (gonocytes) in luminal position, inappropriately expressing OCT3/4 (red staining) well beyond birth, next to more mature spermatogonia at the basal membrane, expressing TSPY (blue cells). OCT3/4 – TSPY double staining, 200×.

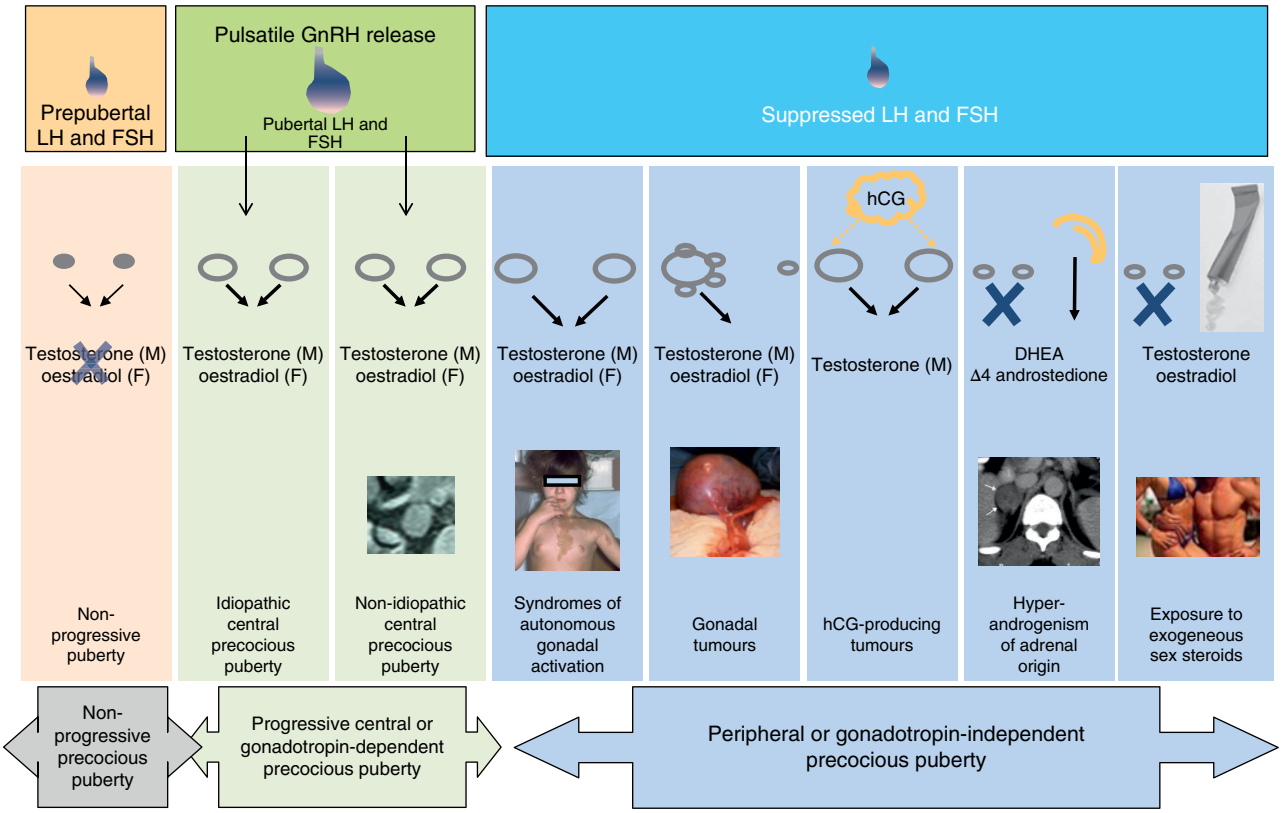

Figure 7.14 Aetiologies of precocious puberty.

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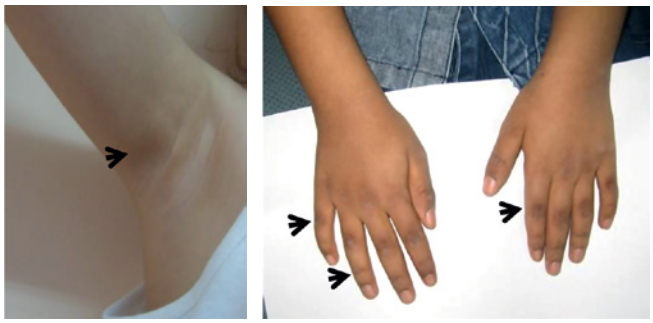

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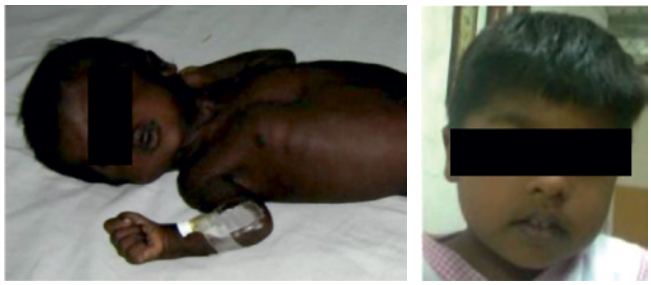

Figure 9.17 Clinical signs of hyperpigmentation associated with primary adrenal insufficiency. (a) Hyperpigmentation of the axilla and knuckles (arrows). Photographs published with consent and courtesy of Professor Lou Metherell. (b) Generalized hyperpigmentation in the newborn period in a baby with ACTH resistance (left panel). The same child is shown after several years of steroid replacement (right panel). (*Source:* Reproduced from Jain et al. [13]. © The Authors under a CC-BY-3.0 licence.)

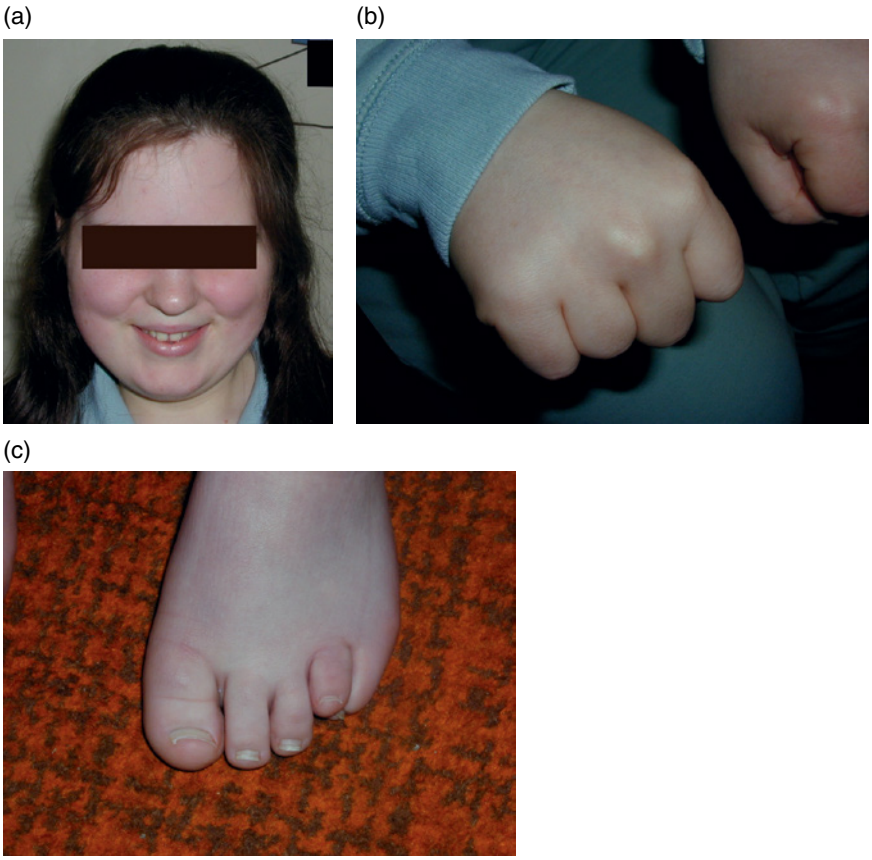

Figure 10.11 Photographs of the face (a), showing the typical rounded facies, and of the right hand (b) and left foot (c) of a patient with pseudohypoparathyroidism type Ia showing the typical shortening of the metacarpals and metatarsals seen as part of Albright's hereditary osteodystrophy (AHO). She had presented with short stature. A mutation in the GNAS1 gene has been demonstrated in this patient. *Source:* Reproduced with the kind permission of the patient.

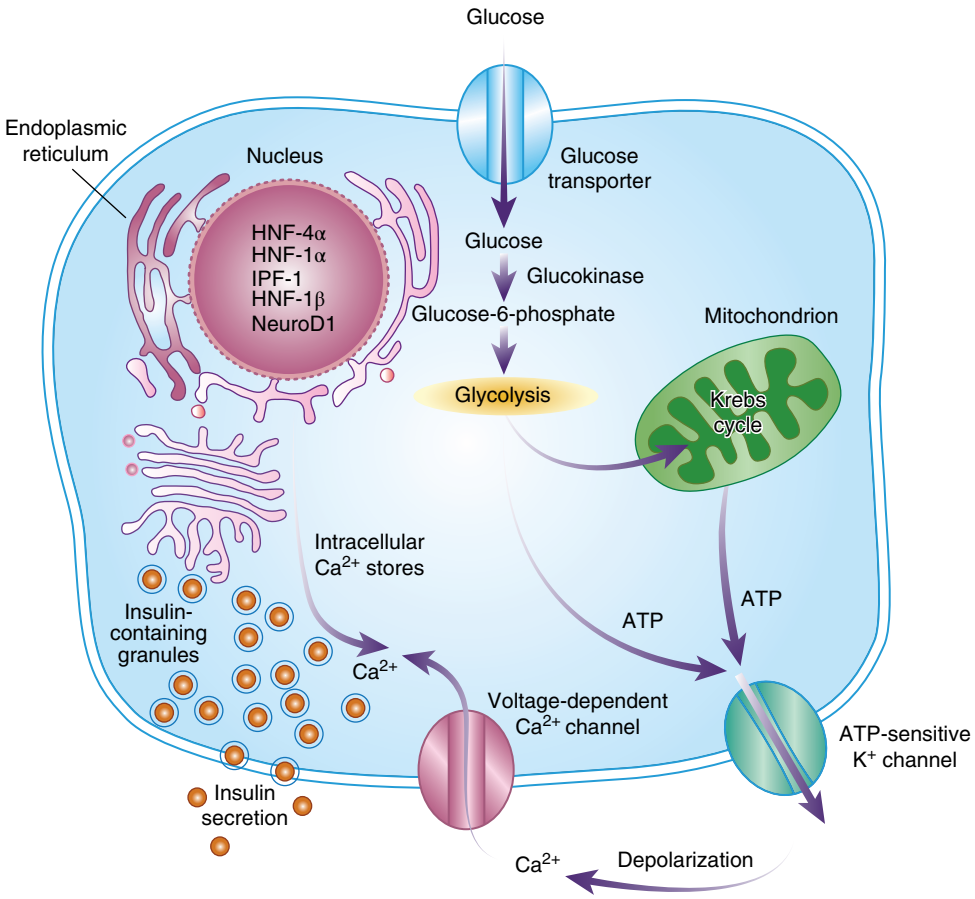

Figure 15.15 Pancreatic β-cell and proteins implicated in MODY pathogenesis [473]. *Source:* Reproduced with permission of John Wiley and Sons.

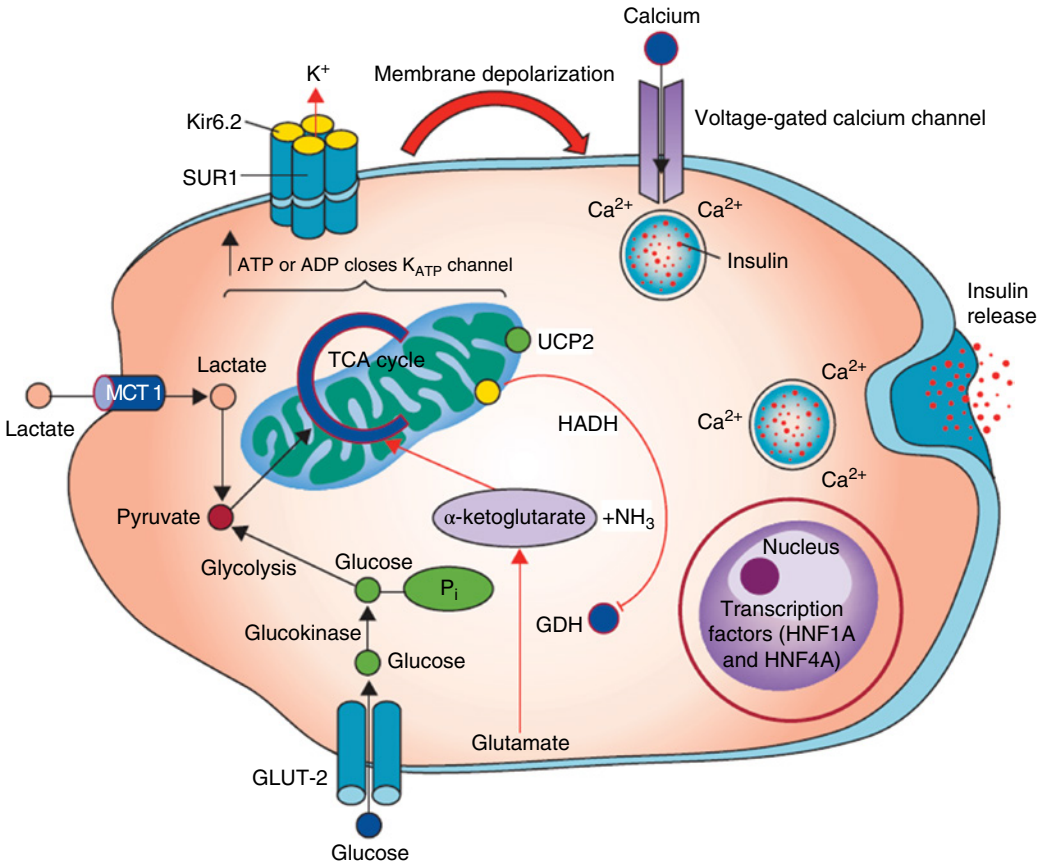

Figure 16.3 Outline of the pancreatic β-cell showing the role of K_{ATP} channels in regulating insulin secretion. β-Cell K_{ATP} channels play a key role in transducing the metabolic signals generated from glucose metabolism to changes in plasma membrane electrical activity and insulin secretion. *Source:* Figure adapted from Shah P et al. [53].

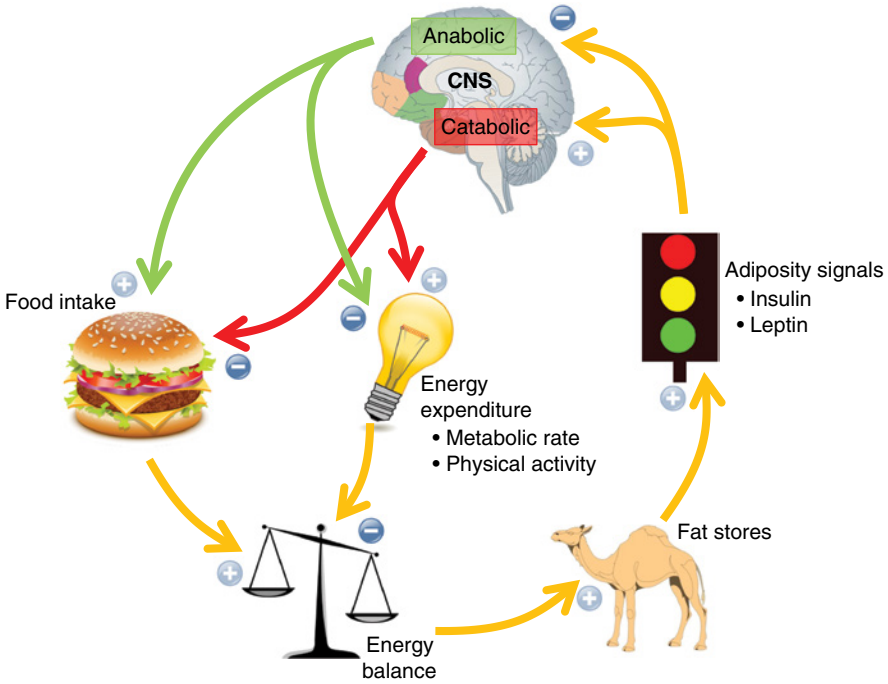

Figure 17.3 Interaction of food intake and energy homeostasis. There is a close interaction between the central nervous system (CNS), food intake, fat storage, adiposity signals, energy expenditure and balance to regulate energy homeostasis.