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ESSENTIALS The inborn errors of metabolism are those inherited diseases in which the phenotype includes a characteristic constellation of bio- chemical abnormalities related to an alteration in the catalytic ac- tivity of a single specific enzyme, activator, or transport protein. Mechanism of diseases—mutations in the proteins giving rise to the inborn errors of metabolism affect primary, secondary, tertiary, or quaternary structure. This can lead to an enormous variety of con- sequences, including (1) abolishing, decreasing, or (occasionally) increasing protein activity; (2) affecting activator proteins, or binding of hormones and other ligands to cell surfaces or other structures; (3) impeding intracellular trafficking and folding of proteins, as well as their post- translational modification through, for example, glycosylation, phosphorylation, or prenylation; (4) affecting the transport of metabolites across cellular membranes; and (5) affecting the formation, activation, or transport of key cofactors for enzymes and other proteins (e.g. vitamins or metal ions). Clinical presentation—the manifestations of metabolic disease are protean and may seem nondescript, especially in adults, hence a high level of suspicion may be required to make a correct diagnosis. Inborn errors of metabolism usually come to light in the neonatal period or infancy, but they may arise even in mature adults for the first time, when their rate of progression may be indolent. Prevention and screening—there is a strong case for mass popu- lation screening for some inborn errors of metabolism at the presymptomatic stage to allow early detection and introduction of proven treatment before irreversible damage occurs. Management—definitive cure of the underlying abnormality is available for a few disorders, but precise characterization of the biochemical disturbance often permits rational treatment to be or- ganized and provides the basis for further therapeutic endeavours. General approaches include (1) restriction of a substrate that cannot be metabolized including molecules derived from the diet; (2) re- placement of a missing metabolic product; (3) removal of poisonous metabolites or rebalancing overproduction of toxic intermediates; (4) administering pharmacological doses of a cofactor, sometimes a vitamin, that

may also stabilize a mutant enzyme; (5) replacement of a missing gene product, usually by enzymatic augmentation therapy or pharmacological chaperones, to prevent premature aggregation and denaturation; (6) repression of an overproduced protein or me- tabolite by stable RNA inhibition (e.g. in transthyretin-induced amyl- oidosis or by supressing 5-aminolaevulinic acid synthase in acute porphyria syndromes); (7) transplantation of cells (e.g. haematopoi- etic stem cells) or organs (e.g. liver) as a ‘gene replacement therapy’; and (8) activation of a poorly functioning protein (e.g. the successful use of an oral activator of a relatively uncommon mutant cystic fi- brosis transmembrane regulator protein such as the G551D missense variant in patients with the cognate disease). Introduction Definition and prevalence The inborn errors of metabolism are those inherited diseases in which the phenotype includes a characteristic constellation of biochemical abnormalities related to an alteration in the catalytic activity of a single specific enzyme, activator, or transport protein. About 1500 such disorders have been characterized with an estimated overall birth frequency of 1 in 1500 live births in nonconsanguineous popu- lations. While these are now recognized as belonging individually to the category of ‘rare diseases’, they can also be viewed as para- digmatic examples of the interplay between the constitutional and environmental aspects of disease as well as selective factors acting over the long course of human evolution. Historical perspective Inborn errors of metabolism were the brainchild of the physician Archibald Garrod in the early 20th century. In the century since their discovery, this brilliant concept has proved to have far-reaching implications in biochemistry, genetics, evolutionary biology, and medical practice. William Bateson, a biologist and early champion of Mendel, showed Garrod that central to his idea of the ‘inborn’ was the concept of the gene. Garrod proposed that genetic deter- minants specify the activity of enzymes which catalyse particular metabolic reactions. Thus Garrod was the first physician and clin- ical biochemist who, with Bateson, applied Mendelian genetics to 12.1 The inborn errors of metabolism:
General aspects Timothy M. Cox and Richard W.E. Watts† † It is with great regret that we report that Richard W.E. Watts died on 11 February, 2018.

 humans: they came to understand the segregation of recessive traits in pedigrees affected by hereditary metabolic diseases and the fre- quent role of consanguinity in very rare disorders. Since Garrod’s original description, the term ‘inborn error’ has broadened to in- clude mutations affecting the function of other proteins, such as the structural proteins fibrillin and collagen as well as transport proteins in which mutations also cause disease. Garrod considered that some ‘simple’ biochemical traits inherited as Mendelian recessive characters, such as alkaptonuria, appeared to have little, if any, apparent effect on health (in fact, in this emblem- atic disorder, the focus of much of his early experimentation, the great man was mistaken: alkaptonuric subjects develop severe arth- ritis, renal and prostatic calculi, and often die prematurely from the consequences of cardiovascular disease (see Chapter 12.2)). In other conditions, such as albinism or porphyria, environmental factors (e.g. sunlight, barbiturates) cooperate with host determinants in the development of clinical manifestations. Thus Garrod promulgated the notion of ‘chemical individuality’ and genetic predisposition to disease; in so doing, he adduced a strong theoretical underpin- ning to the concept of ‘diathesis’—a hitherto pervasive term of the 19th century, largely concealing prejudice and ignorance but long persistent in clinical thinking. Indeed, years after the publication of Garrod’s work, the great American geneticist Thomas Hunt Morgan stated in his Nobel lecture of 1934: I am aware, of course, of the ancient attempts to identify certain gross physical human types—the bilious, the lymphatic, the ner- vous, and the sanguine dispositions, and of more modern attempts to classify human beings

into the cerebral, respiratory, digestive and muscular, or, more briefly into asthenics and pycnics. Some of these are proposed to be more susceptible to certain ailments or diseases than are other types, which in turn have their own consti- tutional characteristics. These well-intended efforts are, however, so far in advance of our genetic information that the geneticist may be excused if he refuses to discuss them seriously. In fact, by 1931 Garrod had developed his ideas in a prescient essay, Inborn Factors in Disease, which has prodigious implications for a modern synthesis of the concept of disease; he had advanced his logic from the inborn error to chemical individuality—a universal quality of the whole species, as opposed to the single individual. It is clear that Garrod had in mind the operation of Darwinian prin- ciples: in the example of infectious disease, he refers to the individu- ality of the human individual and the microbe: In our fight against infective diseases we are not confronted with blind forces, acting at random, but with the disciplined offensive of highly trained foes. Whilst on the one hand the weapons of attack have been improved by evolution, there has been a corres- ponding evolution of protective mechanisms of great ingenuity, and of no small efficiency, for the defence of the individual attacked. To understand the diverse manifestations of disease, including those clearly due to infectious agents such as Mycobacterium tu- berculosis, and variable responses to drugs (many of which were metabolized by enzymes in the liver), Garrod further considered the idea of individual uniqueness and interactions with the envir- onment: he realized that an infinite multitude of responses to en- vironmental factors were determined by constitutional (genetic) variation in the individual and, in effect, that the operation of selection in human evolution is also played out within the micro- cosm of disease. While it has been pointed out that Garrod did not use the terms ‘multifactorial’ or ‘susceptibility’ and had a rudimentary under- standing of genetics, his ideas foresaw the concept of ‘complex’ dis- eases with their dynamic gene–environment interactions. After the more accessible monogenic disorders, the immense technological power available for molecular analysis of the human genome is yielding extraordinary information of both therapeutic and diag- nostic significance. As to the complex diseases, it now seems that the discovery, characterization, and quantification of environmental factors and their interactions with human genetic variants is perhaps the greatest impediment to the mechanistic understanding of these disorders. To summarize: like many others before and after him, the inten- sive study of rare human phenotypes led Archibald Garrod to make observations of astonishing relevance to large fields of medicine. Perhaps the greatest, most penetrating—and lasting—insight to emerge from the concept of the ‘inborn’ has been the realization that disease can no longer be viewed solely in the context of the ‘broken machine’ metaphor, but rather is the consequence of interactions between individual uniqueness and an environment for which that individual is, at a given time, maladapted or ‘unfit’. Although the constraint of space prevents full consideration of this theme, the Darwinian perspective clearly has far-reaching consequences for the teaching and practice of medicine, indeed a new field of evolu- tionary medicine emerges directly (see Chapter 2.2). Classifications of inherited diseases

of metabolism Almost all the inborn errors of metabolism arise from mutations in the nuclear genome and have Mendelian patterns of inheritance, but 13 genes are encoded by the mitochondrial genome, and when these are mutated the cognate diseases are maternally transmitted. Mutations in the proteins giving rise to the inborn errors of me- tabolism affect primary, secondary, tertiary, or quaternary structure. These can lead to an enormous variety of consequences, including (1) abolishing, decreasing, or (occasionally) increasing protein ac- tivity; (2) affecting activator proteins, or binding of hormones and other ligands to cell surfaces or other structures; (3) impeding intra- cellular trafficking and folding of proteins, as well as their post-

translational modification through, for example, glycosylation, phosphorylation, or prenylation (a post-translational modification in which a isoprenyl group is added to a cysteine residue—a process which mediates protein interactions, especially protein–membrane interactions); (4) affecting the transport of metabolites across cel- lular membranes; and (5) affecting the formation, activation, or transport of key cofactors for enzymes and other proteins (e.g. vita- mins or metal ions). The complex genome The complexity of the human genome is becoming ever more ap- parent, with recent findings from a project called the Encyclopedia of DNA Elements (ENCODE) confirming that, contrary to previous supposition, most of the 3 billion base pairs that it contains have a function. Regulation of gene function is proving to be much more

12.1 The inborn errors of metabolism: General aspects 1931 intricate that at first thought. There are nearly 20 000 genes that en- code proteins, and DNA sequences also encode thousands of add- itional RNA molecules. Of the greater than 11 000 DNA sequences classified as pseudogenes, several are now known to be active in some cell types or individuals. We now know that genes can overlap and may have multiple start and termination points. ENCODE has uncovered 4 million short stretches of DNA that control gene ac- tivity, which can act combinatorially and in different cell types to give each a unique identity. Some of the RNA strands (transcribed as microRNAs) also influence gene expression. In a reference to the lexicon of human genetics (Mendelian Inheritance in Man), there are estimated to be 24 600 potential human phenotypes and 8670 known or suspected Mendelian dis- eases, the inheritance of which can be described as being auto- somal recessive, autosomal dominant, sex-linked, or transmitted maternally through the mitochondrial genome (Online Mendelian Inheritance in Man, http://www.ncbi.nlm.nih.gov/Omim/, http://www.omim.org/statistics/entry as of 27 June 2018). The inborn errors of metabolism are those inherited diseases in which the phenotype includes a characteristic constellation of chemical abnormalities related to an alteration in the catalytic activity of a single specific enzyme, activator, or transport pro- tein. There are unifactorially inherited diseases in which the cur- rent techniques are too insensitive for a chemical abnormality to be identified, so that the syndrome has to be defined in clinical, gross structural, and/or pathological terms; further study is likely to demonstrate that many of these fall into the category of inborn errors of metabolism. Almost all the so-called single-gene diseases arise from muta- tions in the nuclear genome. A few mitochondrial proteins have their structures encoded in the mitochondrial DNA (mtDNA). This genetic information is transmitted only through the female line and maternal inheritance inborn errors of metabolism in- clude mitochondrial diseases. The nuclear and the maternally in- herited diseases stem from mutations of DNA which directs the synthesis of a specific polypeptide chain. The molecular changes in the enzyme protein may affect the primary, secondary, tertiary, or quaternary structure, decreasing, increasing, or abolishing its catalytic activity. Some mutations affect the function of an acti- vator protein, others reduce the binding of hormones and para- crine factors to cell surfaces and/or subcellular structures, and some derange the migration of proteins within cells; another group impairs the transport of metabolites across cellular and subcellular membranes (Table 12.1.1). Most intracellular enzymes are lo- cated in the cytosol where they are correctly orientated in relation to one another, sometimes as macromolecular complexes, and to their substrates. Some are linked to cellular membranes and sev- eral are located in anatomically defined subcellular structures or organelles: the Golgi apparatus, mitochondria, lysosomes, and peroxisomes. Mitochondrial diseases The mitochondrial genome is a circular double strand containing 16.5 kb of DNA. It encodes 13 of the polypeptide subunits of re- spiratory chain enzymes, the remainder of which (c.60) are encoded in the nuclear DNA. Hitherto, mutations in 26

genes in the mito- chondrial genome are associated with defined human phenotypes (see Chapter 24.19.5). Abnormal mitochondrial function impairs the supply of energy for biochemical work in all tissues and there- fore has wide-ranging effects. Each mitochondrion also contains 24 RNA genes that participate in intramitochondrial protein synthesis. Transcription and translation of mtDNA are regulated by the nu- cleus through interactions with the noncoding D-loop region of the mitochondrial genome. Human cells contain about 1000 copies of mtDNA, but the individual mitochondria in a cell may not all carry a given specific mutation and different cells carry different propor- tions of mutated mitochondria (heteroplasmy). The proportion of mutant mtDNA must exceed a critical level before the mitochon- drial respiratory chain disease declares itself. This variability, as well as tissue-specific differences in dependence on oxidative metab- olism, explains, at least partially, why some tissues are preferentially affected in patients with mtDNA diseases. Table 12.1.1 Examples of diseases in which there is defective transport of an enzyme or metabolite within cells or across cell membranes Disease Metabolic abnormality Cystinuria Failure to transport cystine, lysine, ornithine, arginine, and homoarginine across the plasma membrane of the proximal renal tubular epithelium and the small intestinal mucosa I (inclusion body)-cell disease (mucolipidosis II/III) Failure to generate the key molecular recognition signal, mannose 6-phosphate, on nascent lysosomal glycoproteins that allows them to bind and enter the organelle via mannose 6-phosphate receptors. Multiple acid hydrolases are mistargeted and deficient in lysosomes, leading to build up of undegraded macromolecules (as inclusions). The disease is due to defective action of N-acetylglucosamine-1-phosphotransferase—a heteromeric complex of polypeptides that are the products of two genes Cystinosis (cystine storage disease, Lignac’s disease) Failure to transport cystine produced by intralysosomal proteolysis across the lysosomal membrane and into the cytosol Salla disease Failure to transport N-acetylneuraminic acid (sialic acid) across the lysosomal membrane and into the cytosol Mucopolysaccharidoses Failure to degrade glycosaminoglycans (mucopolysaccharides), the undegraded mucopolysaccharides are neither transportable across lysosomal membranes nor capable of being removed from the lysosomes by exocytosis Tay–Sachs disease Defective post-translational processing of the α chain of β-N-acetylhexosaminidase (hexosaminidase A) in some mutants. This prevents the enzyme from migrating from the endoplasmic reticulum, where it is glycosylated, to the Golgi apparatus for phosphorylation of its mannosyl residues and hence to lysosomes and the exterior of the cell Primary hyperoxaluria type 1 (some cases) Mislocation of alanine: glyoxylate aminotransferase in mitochondria as opposed to its normal location in peroxisomes. This arises because a rare mutation (Gly 170 → Arg) is present simultaneously with the common polymorphism (Pro 11 → Leu). The mutation (Gly 170 → Arg) prevents dimerization of the molecule which, in turn, allows the weak mitochondrial targeting sequence generated by the polymorphism (Pro 11 → Leu) to direct the molecule to mitochondria instead of peroxisomes

 Postmitotic tissues (e.g. neurons, muscle, and endocrine tis- sues) have high levels of mutated mtDNA and are often clinically affected, whereas rapidly dividing tissues (e.g. bone marrow) tend to be less often clinically affected. Differences in the proportions of mutated and nonmutated mtDNA between and within family mem- bers also contribute to the wide phenotypic range encountered in the mitochondrial diseases. The spermatozoal cytoplasm, including its mitochondria, is entirely lost at fertilization and for this reason mitochondrial diseases are only transmitted through the female line. Clinically affected women rarely transmit a mtDNA deletion to their children. However, a woman with a heteroplasmic mtDNA point mutation or duplication may transmit a variable amount of mutated mtDNA to her progeny. The number of

mtDNA molecules in each oocyte is reduced and then amplified to a total of about 105 during early development of the oocyte; this presumably random process contributes to the different amounts of mutated mtDNA in different children in the same family. Women whose gametes contain high concentrations of mtDNA are more likely to have clinically affected children than mothers with lower concentrations of mtDNA. The general clinical manifestations of the mitochondrial diseases are shown in Table 12.1.2 and specific examples of mitochondrial dis- eases are given in Box 12.1.1. Peroxisomal diseases Some enzymes and other proteins that are encoded in the nuclear DNA are specifically expressed in peroxisomes, to which they are imported soon after translation. Mutations in these genes result in the peroxisomal diseases listed in Box 12.1.2. Diseases due to de- fects in peroxisomal proteins are discussed in Chapters 12.9, 12.10, and 24.17. Lysosomal diseases Lysosomes are subcellular organelles containing hydrolases with low optimum pH values (‘acid hydrolases’) which catalyse the degrad- ation of cellular macromolecules. The macromolecules are either derived from the metabolic turnover of structural cellular compo- nents or have entered the cell by endocytosis. The products of this macromolecular degradation process leave the lysosomes by specific efflux processes. Table 12.1.2 The main clinical manifestations of diseases due to mitochondrial dysfunction Disease group Clinical manifestations Defects of fatty acid oxidation Hypoglycaemia Hepatic dysfunction Cardiac failure Myopathy Sudden infant death Respiratory chain disorders Lactic acidosis Encephalopathy Hypotonia Poor feeding Failure to thrive Convulsions Box 12.1.1 Some mitochondrial diseases Mitochondrial DNA defects Rearrangements (deletions and duplications) • Chronic progressive external ophthalmoplegia • Kearns–Sayre syndrome (hypoparathyroidism with deafness) • Diabetes and deafness Point mutations in protein encoding genes • Leber’s hereditary optic neuropathy • Leber’s hereditary optic neuropathy/dystonia • Neurogenic weakness, ataxia, and retinitis pigmentosa • Leigh’s syndromea Point mutations in tRNA genes • Mitochondrial encephalopathy with lactic acidosis and stroke-like episodes • Myoclonic epilepsy with ragged-red fibres • Myopathy • Cardiomyopathy • Diabetes and deafness • Encephalomyopathy • Leigh’s syndromea Point mutations in rRNA genes • Nonsyndromic sensorineural deafness • Aminoglycoside-induced nonsyndromic deafness Nuclear DNA defects Nuclear genetic disorders with a mitochondrial basis • Friedreich’s ataxia (frataxin) • Autosomal recessive hereditary spastic paraplegia Nuclear genetic disorders of the mitochondrial respiratory chain • Leigh’s syndrome (complex I deficiency)a • Optic atrophy and ataxia • Leigh’s syndrome (complex IV deficiency)a Nuclear genetic disorders associated with multiple mtDNA deletions • Autosomal dominant external ophthalmoplegia • Mitochondrial neurogastrointestinal encephalomyopathy (thymidine phosphorylase deficiency) a An example of different mutations providing the same clinical syndrome (phenocopies). mtDNA, mitochondrial DNA; rRNA, ribosomal RNA; tRNA, transfer RNA. Data from Chinnery PF and Turnbull DM (1999) The Lancet 354 (supplement 1), S17–S21. Box 12.1.2 Peroxisomal diseases • Zellweger’s syndrome (absent peroxisomal membranes) • Pseudo-Zellweger’s syndrome • Adrenoleukodystrophy • Pseudo-neonatal adrenoleukodystrophy • Acatalasia • Infantile Refsum’s disease • Refsum’s disease (classical form) • Hyperpipecolic acidaemia • X-linked adrenoleukodystrophy • Chondrodysplasia punctatum rhizomelia • Primary hyperoxaluria type 1

 In most of the lysosomal storage diseases, an inborn error of metabolism affects a specific lysosomal enzyme so that either undegraded or partially degraded macromolecules accumulate in the lysosomes (see Chapter 12.8). The engorged lysosomes distort the internal architecture of the cell, disturb its function, and in- hibit the activities of other lysosomal enzymes so that macromol- ecules other

than those related to the primary enzyme deficiency also accumulate. Cystinosis (cystine storage disease) and Salla disease (N- acetylneuraminic (sialic) acid storage disease) are due to metabolic lesions involving the specific efflux processes by which these small molecules generated by the intralysosomal hydrolysis of complex substrates cystine and sialic acid, respectively, leave the lysosome (Table 12.1.1). Lysosomal enzymes are glycoproteins which are subject to exo- cytosis and reuptake by endocytosis. Their protein moieties are synthesized on the rough endoplasmic reticulum and the oligosac- charide side chains are added in the Golgi apparatus. The addition of a terminal mannose 6-phosphate residue recognition marker is necessary if the enzyme molecule is to be correctly routed into the lysosomes, and if it is to be available for receptor-mediated re-uptake from the interstitial fluids. The types of lysosomal storage diseases and the nature of their metabolic defects together with examples of each group are presented in Table 12.1.3.
Heterogeneity in the inborn errors of metabolism The individual inborn errors of metabolism are defined on the basis of the phenotype, including the specific enzyme lesion, and by their pattern of inheritance. Close study of any particular in- born error of metabolism reveals unexpected heterogeneity and we are increasingly recognizing diverse patterns of inheritance due to a variety of mechanisms, including somatic mosaicism, dominant negative effects in complex multimeric pathways, as well as transcriptional silencing of imprinted genes. This may be due to: • multiple allelism • mutations at different gene loci affecting the structure of different polypeptide chains in a single enzyme protein • mutations at different gene loci affecting different proteins with similar catalytic functions • differences in the overall genetic background against which the single mutation acts • environmental factors. Table 12.1.3 Lysosomal storage diseases other than cystinosis and Salla disease; a more complete listing is given in Chapter 12.8 (Table 12.8.1) Name Defect Example Sphingolipidoses Failure to degrade compounds containing a sphingoid (sphingolipids, ceramides, sphingomyelins, and glycosphingolipids including the gangliosides (sialoglycosphingolipids)) Tay–Sachs disease (GM2-gangliosidosis) Gaucher disease (glucocerebrosidosis) Mucopolysaccharidoses Failure to degrade the glycosaminoglycans: dermatan, heparan, and keratan sulphates. Incompletely degraded glycosaminoglycan fragments accumulate in the lysosomes as well as extracellularly. This causes secondary deficiencies of other lysosomal enzymes and other undegraded macromolecules, particularly sphingolipids, accumulate Hurler’s disease Hunter’s disease Morquio disease (MPS 1, 2, 4) Glycoproteinoses A group of enzyme defects in the catabolism of glycoproteins in which characteristic abnormal macromolecules accumulate Fucisidosis Mannosidosis Acid lipase deficiency Two clinically distinct variants. Cholesteryl esters and triglycerides accumulate in most tissues due to deficiency of lysosomal acid lipase Wohlman’s disease Cholesteryl ester storage disease Glycogenosis II Lack of intralysosomal hydrolysis of glycogen Glycogenosis type II (only member of this group) Mucolipidoses Originally defined as being clinically intermediate between the sphingolipidoses and mucopolysaccharidoses but without mucopolysaccharidu ria (abnormal glycosaminoglycan excretion). Subsequently shown to include patients with (1) deficient neuraminidase activities with respect to either glycoprotein substrates (mucolipidosis I, also classified as a glycoproteinosis and termed sialidosis) or ganglioside substrates (mucolipidosis IV); (2) clinically mild and severe variants of uridine-diphosphate-N-acetylglucosamine: lysosomal enzyme precursor N-acetylglucosamine phosphate transferase (mucolipidoses II (I-cell disease) and III (pseudo-Hurler polydystrophy) respectively—see Table 12.1.1) See text Miscellaneous Numerous defects affect the action or activation of lysosomal enzymes—sulphatase-modifying factor, which is critical for a post-translational modification of a common cysteine residue in eight of 18 putative human sulphatases; the four sphingolipid activator proteins, A–D, and GM2 activator protein, which

act as enzymatic activators in multiple stages of lysosomal sphingolipid degradation, as well as lysosomal membrane digestion Deficiency of the lysosomal integral membrane protein, LIMP-2, associated with selective deficiency of glucocerebrosidase in nonmacrophage lineage cells and action myoclonus–renal failure syndrome. Mutations in the X-chromosome-linked LAMP-2 lysosomal membrane protein cause Danon's disease with defective clearance of autophagic debris, including glycogen, principally affecting heart, skeletal muscle, and the brain Multiple sulphatase deficiency GM2 gangliosidosis (AB variant) Action myoclonus–renal failure Danon's disease

SECTION 12 Metabolic disorders 1934 General approaches to the management of inborn errors of metabolism Clinical indicators of an inborn error of metabolism The manifestations of metabolic disease are protean and may seem nondescript, especially in adults. Some clinical settings suggest the presence of an inborn error of metabolism (Box 12.1.3), but in other circumstances a high level of suspicion may be required to make a correct diagnosis. Inborn errors of metabolism usually come to light in the neonatal period or infancy, but can occur at any time—even in mature adults for the first time and in whom the rate of progression may be indolent. In an appropriate clinical context— for example, unexplained acute neonatal illness and/or failure to thrive in early infancy, developmental slowing and arrest followed by retrogression, or unusual physiognomy—the critical clue often comes from taking an appropriate family history, with specific in- quiries about affected siblings, possible parental consanguinity, pa- ternity, miscarriages, perinatal deaths, abortions, about the sexes of possibly affected relatives and their placement on the maternal or paternal side of the family, the ages at death of relatives, as well as the ethnic and geographical origins of the parents. Impaired func- tion of proteins that are localized to the mitochondria, lysosomes, and peroxisomes are associated with particular clinical and bio- chemical characteristics that reflect the compartmentalized func- tions of these organelles. A broad perspective of the physician's role Inborn errors of metabolism encompass diseases that are disabling, disfiguring, and painfully life-shortening, hence—as in many areas of medicine—the role of the physician is much broader than dis- pensing the perceived and much-awaited 'magic' of a cure. From the clinical aspect, often the best achievable goals are preventing rapid deterioration and ensuring that emergency measures are in place so that acute metabolic decompensation can be quickly contained, with preservation of tissue integrity and organ function, especially of the brain. The time-honoured adage: 'to cure sometimes, to re- lieve often—but comfort always' is particularly telling in this field of practice. It is also important to emphasize the critical importance of prompt diagnosis—even of what might prove to be an incurable disease—together with the mandatory requirement for providing genetic advice. Principles of treatment While many inborn errors of metabolism have severe and poten-tially lethal effects, as a group, effective treatments are continually being introduced. Therapeutic advances are increasingly based on a scientific understanding of the inherited defect, to which bio chemical knowledge can be rationally applied. The treatments available for the individual disorders are diverse and often must be specially developed for individual patients. General approaches include (1) restriction of a substrate that cannot be metabolized including molecules derived from the diet; (2) replacement of a missing metabolic product; (3) removal of poisonous metabol- ites or rebalancing overproduction of toxic intermediates; (4) ad- ministering pharmacological doses of a cofactor, sometimes a vitamin, that may also stabilize a mutant enzyme; (5) replacement of a missing gene product, usually by enzymatic augmentation therapy or pharmacological chaperones, to prevent premature aggregation and denaturation; (6) repression of an overproduced protein or metabolite by stable RNA inhibition; (7) transplant- ation of cells (e.g. haematopoietic stem cells) or organs (e.g. liver) as a 'gene replacement therapy'; and (8) activation of a poorly functioning

protein. The principles involved are summarized in Table 12.1.4. While dealing with the complexities of each individual disorder, the essence of clinical care for these chronic disorders requires maturity, vigilance, and the perspective of the generalist in paedi- atric or adult medicine to ensure provision of what is now termed holistic medicine. Palliative surgical and other measures may be needed to deal with specific complications (e.g. corneal grafting to restore vision in patients with corneal clouding due to one of the mucopolysaccharidoses). Consideration should also be given to meeting the educational and social needs of these patients as well as to optimizing their overall clinical state and correcting the biochem- ical parameters. Beyond the skills of other physicians, surgeons, biochemists, and geneticists, successful management of patients with inborn errors of metabolism requires multidisciplinary engagement by colleagues Box 12.1.3 Clinical presentations which, in the absence of acquired or other congenital causes, suggest an inborn error of metabolism • Unexplained acute neonatal illness and/or failure to thrive in early in- fancy. (Marked muscle hypotonia, recurrent fits, comas, acidosis, and vomiting, especially if withholding milk feeds causes temporary im- provement, are especially suggestive) • Developmental slowing and arrest followed by retrogression • Developmental slowing and arrest leading to unexplained intellectual disability • Unusual physiognomy, multiple skeletal deformities with develop- mental delay and retrogression • Multiple skeletal deformities alone (dysostosis multiplex especially suggests a lysosomal storage disease) • Gross visceromegaly • Specific dietary intolerances • Haemolytic anaemia • Unusual body odoura • Urolithiasis • Cataracts in early lifeb • Dislocation of the optic lensc • Persistent jaundice and hepatic cirrhosis in infancy. • Abnormal cutaneous photosensitivity • Hypopigmentation • Abnormal drug sensitivity • A history of recurrent perinatal deaths and/or stillbirths • Hydrops fetalis in the absence of blood group incompatibility between mother and fetus (red cell enzyme defects) a Examples are phenylketonuria (mousy, musty), branched chain ketoacidosis (maple syrup), methionine malabsorption (oast house, dry celery), isovaleric acidaemia (sweaty feet), trimethylaminuria (stale fish), multiple carboxylase deficiency (tom cat’s urine), and hawkinsinuria (swimming pool). b Examples are Fabry’s disease, galactosaemia, galactokinase deficiency, Lowe’s syndrome, mannosidosis, osteogenesis imperfecta, Refsum’s disease, and Wilson’s disease. c Examples are Ehlers–Danlos syndrome, homocystinuria, hyperlysinuria, Marfan’s syndrome, and sulphite oxidase deficiency.

 with special skills related to metabolic medicine—including diet- itians, social workers, educationalists, and occupational therapists. It is particularly important to plan for the handover of specialist care from the paediatrician to the most appropriate adult physician when follow-up in a paediatric department becomes inappropriate. The perfect outcome is to achieve a physically and mentally healthy and fulfilled adult who is capable of begetting healthy children. Unfortunately, the nature of many of the inborn errors militates against this ideal so treatment has to aim at optimizing the child’s potential in all its physical, mental, and social aspects. Treatment and support also have to be extended to the parents and siblings who, if not overtly affected themselves, may be carriers of the ab- normal gene concerned and require appropriate advice about the transmission of the disease to other offspring and other aspects of the condition. Particular treatments Protein replacement therapies The ability to clone human genes into bacteria and eukaryotic cells for ectopic expression which can then produce large amounts of the human gene product is opening the horizons for treatment by protein replacement or more accurately, augmentation. The devel- opment of macrophage-targeted β-glucosylceramidase enzyme re- placement therapy for Gaucher’s disease is a notable development in this field and is now regarded as the definitive treatment for the non-neuronopathic

manifestations. Attempts to utilize transplanted fibroblasts and amniotic cells as a source for enzyme replacement therapy have not been successful. Bone marrow transplantation (‘haematopoietic stem cell transplant- ation’) has been used for the treatment of two groups of inherited Table 12.1.4 General approaches to the treatment of inborn errors of metabolism Method Examples General measures (directed to mitigate) Ultraviolet radiation (congenital erythropoietic and variegate porphyrias, and in albinism) Ionizing radiation in the DNA repair enzyme defects (xeroderma pigmentosum, ataxia telangiectasia) Infections (agammaglobinaemia). Restriction of a dietary substrate which cannot be metabolized Partial inhibition of formation of toxic metabolites (inhibitors of biosynthesis) Phenylalanine restriction in phenylketonuria Protein restriction in the hyperammonaemias Elimination of galactose in galactosaemia Restriction of dietary phytanic acid and congeners (Refsum’s disease) Medications (oestrogens, barbiturates, etc. in acute intermittent porphyria) statins (hypercholesterolaemia); haem arginate (acute porphyrias); nitisinone (hereditary tyrosinaemia type I); allopurinol (gout); eliglustat (type I Gaucher disease) Supplying a missing metabolic product Orotic aciduria: treatment with uridine triacetate which is metabolized to uridylic acid Hartnup disease: nicotinic acid to control pellagra Removal of toxic metabolite Haemodialysis and peritoneal dialysis as temporary treatment of an acute metabolic crisis due to a diffusible toxic metabolite, and to correct certain secondary biochemical abnormalities quickly Either specific chemical detoxication (e.g. penicillamine in Wilson’s disease) or solubilization (e.g. penicillamine in cystinuria); accelerated metabolic disposal (phenylbutyrate and sodium benzoate in hyperammonaemia due to urea cycle defects) Pharmacological doses of a cofactor (only some cases of each disease respond). Note: this stratagem has features of pharmacological chaperone therapy since natural cofactors such as vitamins also stabilize mutant enzymes Propionic acidaemia: biotin Ubidecarenone (respiratory chain disorders due to coenzyme Q10 deficiency) Homocystinuria: pyridoxine Primary hyperoxaluria (type I): pyridoxine Methylmalonic acidaemia: vitamin B12 and other cobalamins Replacement of a missing gene product Adenosine deaminase deficiency Gaucher disease: β-glucosylceramidase (mannose-terminated to confer selective uptake by tissue macrophages) Haemophilia: clotting factor VIII Activating a membrane transporter Cystic fibrosis (due to G551D mutation—a relatively uncommon variant of the cystic fibrosis transmembrane regulator protein, CFTR, a chloride channel) Bone marrow transplantation Adenosine deaminase deficiency; infantile Krabbe’s disease; α-mannosidosis Haematopoietic stem cell transplantation Adenosine deaminase deficiency Liver transplantation Hereditary tyrosinaemia (type I) Antitrypsin deficiency Primary hyperoxaluria (type I) Urea cycle disorders Crigler–Najjar syndrome (type I) Gene replacement RNA interference therapy (siRNA) Adrenoleukodystrophy adenosine deaminase deficiency Patisiran for transthyretin-related polyneuropathy (systemic amyloidosis) Note: the examples chosen are situations in which either the proposed treatment is established or in which it can be recommended as elective therapy even though the results of prolonged evaluation are still awaited.

 metabolic disorders: those in which it is desired to replace a par- ticular type of nonfunctioning bone marrow cell by its normally functioning counterpart, and those in which an attempt has been made to utilize the fact that the bone marrow produces leucocytes and that these cells exocytose (release) their lysosomal enzymes for endocytic uptake by enzyme-deficient cells in the body tissues generally. Bone marrow transplantation has been more successful with the first group of diseases, which includes disorders of neutrophil func- tion (e.g. cyclic neutropenia), functional abnormalities of lympho- cytes, and osteopetrosis; the beneficial effect on the latter being due to the introduction of normal osteoclast precursors derived

from granulocyte–macrophage progenitors in the marrow. The results of bone marrow transplantation in the second group of diseases, namely those in which the white cell lineage derived from the transplanted bone marrow is used to supply normal en- zyme to enzyme-deficient tissues, for example, Hurler's disease (mucopolysaccharidosis type 1) and Krabbe's disease, has been less effective. In the latter case, recent courageous studies have shown that transplantation of HLA-matched umbilical cord blood in the first 10 days of life has been moderately successful in promoting neurological development in infants born to couples with previously affected offspring and detected by screening early for this otherwise rapidly progressive neurodegenerative disorder. Haematopoietic stem cells have been implanted into the fetus in utero to correct se- vere congenital immunodeficiency, but this has not, so far, been ap- plied to diseases without immunodeficiency. This procedure takes advantage of the immunological tolerance of the fetus. The possibility of using liposomes and resealed erythrocyte en- velopes as carriers of therapeutic enzymes is also being explored; linking purified or recombinant therapeutic enzymes such as adeno- sine deaminase to polymers such as polyethylene glycol may usefully prolong their survival in circulating plasma. Receptor-targeted therapies Definitive enzymatic augmentation with receptor-targeted therapies has attracted much attention. In Gaucher's disease, this strategy has proved to be very effective and commercially successful: global sales of the mannose-terminated glucocerebrosidase for about 6000 patients worldwide enabled the Genzyme corporation to rise to a leading pos- ition in the biotechnology industry. Other industrial competitors have followed suit with targeted enzyme preparations approved for this and other lysosomal diseases such as Fabry's disease, Pompe's disease, and mucopolysaccharidosis types I and II (see Chapter 12.8). Substrate-reduction therapies Substrate-reduction therapy with the use of specific inhibitors to regulate the flux through impaired degradative pathways, by partial blockade of the rate-limiting step is useful in low-density lipoprotein receptor deficiency (heterozygous familial hypercholesterolaemia as well as the very rare homozygous variant), and thus was born the pharmaceutical star of the statin drugs, which are in wide general use. An analogous approach involving inhibition of the first committed step in the biosynthesis of glycosphingolipids is already showing promise in the glycosphingolipid diseases such as Gaucher's dis- ease. Small-molecule inhibitors of this pathway that are safe, well tolerated, and that penetrate the blood–brain barrier have the po- tential to improve the outcome for many patients with progressive neurological complications of the sphingolipidoses, who would otherwise be without hope. Currently two such agents are in active clinical development. In alkaptonuria, a disease in which Garrod maintained a lifelong interest, the use of substrate-reduction therapy is also far advanced. Nitisinone, a triketone inhibitor of the precursor to homogentisic acid at the level of hydroxyphenylpyruvate dioxygenase in the tyro- sine degradation pathway, is a licensed agent for tyrosinaemia type 1. In very small doses, this agent has a striking effect on the forma- tion of toxic oxidative metabolites of homogentisic acid which lead to the life-shortening manifestations of alkaptonuria, and it appears likely that at last a well-tolerated and definitive treatment for this landmark disorder is within sight.
Pharmacological chaperones The concept of pharmacological chaperones, based on the ability of small molecules to bind to mutant proteins to prevent their inactiva- tion by abnormal folding, intracellular aggregation, and mistargeting, is receiving much attention. It has yet to be adopted extensively in practice, although the chaperone approach is in late-phase clinical de- velopment in Fabry's disease and is being explored in Pompe disease. Organ transplantation Liver transplantation is used as a form of functional complementa- tion in some inborn errors of metabolism such as glycogen storage disease type I and severe recurrent acute porphyria where this organ is the specific site of the metabolic lesion. Liver transplantation has the advantage that

the enzyme is introduced in the correct organ, in the correct cell with its correct subcellular location, and correctly orientated with respect to its substrate and other enzymes with which it must act in concert, for example, in the urea cycle disorders such as ornithine transcarbamylase deficiency. Liver transplantation can also be regarded as a form of gene re- placement therapy in that the donor liver contains the normal gene which will direct the synthesis of a normal enzyme protein. Prenatal transplantation of fetal liver stem cells has potential in the treatment of some inborn errors of metabolism. Successful engraftment at the 12th to 24th week after fertilization with partial correction of the metabolic defect has been demonstrated in β-thalassaemia. Gene replacement and cell-based therapies Gene therapy and cell-based therapies including bone marrow transplantation are at various stages of clinical evaluation and devel- opment, assisted by the recent capacity to develop credible models of specific disorders in genetically modified animals. Haematopoietic stem cell gene therapy Gene replacement using retroviral vectors and gene constructs can be used to introduce the desired DNA sequence into the patient's explanted haematopoietic stem cell genome. These genetically cor- rected cells are cultured and then returned to the patient's circula- tion, where they may have therapeutic potential in diseases where expression of the metabolic lesion in the haematopoietic system determines the phenotype or in those situations where genetic- ally corrected migratory cells of haematopoietic origin can deliver normal enzyme to the enzyme-deficient tissues. This approach has recently been reported in young patients with metachromatic leukodystrophy, with the phase I/II clinical trial results indicating

12.1 The inborn errors of metabolism: General aspects 1937 convincing evidence of some neurological benefit or 'rescue' com- pared with historical and family control patients not so treated, and retrospectively with patients treated by transplantation of haem- atopoietic stem cells from healthy donors. Despite the difficulty in determining efficacy directly in such studies, the disease did not manifest or progress in the three patients 7 to 21 months beyond the age at which this would have been predicted. Somatic cell gene therapy Although somatic cell gene therapy using viral vectors and/or gene constructs to introduce the desired DNA sequences into other cell types is currently being investigated extensively in in vitro model systems and in animal models of some human inborn errors of me- tabolism (e.g. using hepatocytes), few of these have reached appli- cation in clinical practice. However, this approach, using lentiviral vectors which have the advantage that they can be used to transduce by nuclear integration of viral sequences in mitotic cells, has had qualified therapeutic success in trials in children with combined immunodeficiency. Several patients in this Anglo-French trial un- fortunately developed a late-onset T-cell lymphocytosis leading to leukaemia, later shown to be related to the integration of vector sequences at a genomic 'hot spot' leading to activation of a neigh- bouring endogenous proto-oncogene. These patients responded to antileukaemic chemotherapy with satisfactory control of the complication— with continued amelioration of their disabling im- munodeficiency disease—but safety considerations have retarded clinical development until improved vector systems can be utilized. Promising results of a gene therapy trial using a lentiviral vector to correct the enzymatic abnormality in leucocytes derived from haematopoietic precursors in a very rare immunodeficiency disease, adenosine deaminase deficiency, have also been reported—so far with no mutagenic effects. Eight years after the procedure, eight of ten patients with severe combined immunodeficiency no longer re- quired enzyme-replacement therapy and lived normally. Gene therapy with autologous CD34+ haematopoietic stem cells transduced with a third generation lentiviral vector reduced or elimin- ated the need for long-term red-cell transfusions in 22 patients

with se- vere β-thalassemia without serious adverse events related to this vector. Given the challenges of donor availability and the added risks of allo- geneic haematopoietic stem-cell transplantation in this disease and the prior occurrence of neoplastic change or of preferential integration of vector at specific sites in the host genome, early regulatory approval of this stratagem, is encouraging for numerous inborn metabolic diseases. The possibility of using adeno-associated viral vectors as a means of introducing corrected genes for into nonmitotic cells of the nervous system is being explored in human patients; these vectors are main- tained as episomal elements which do not integrate readily into the host genome (with the attendant risk of mutagenesis) but persistently express the corrective protein. Adeno-associated vectors have been successfully used in early gene therapy trials of the retinal disease, Leber's congenital amaurosis, with direct intraorbital gene delivery. Recombinant adeno-associated viral vector serotype 9, which supplies the deficient Smn1 protein, was approved by the FDA as Zolgensma after a phase 3 clinical trial in infants less than 2 years of age with spinal muscular atrophy—including the pre-symptomatic phase. The agent prolongs event-free survival and increases motor function. Salutary outcomes in rapidly progressive genetic diseases open up the field for further exploration of gene therapy in the human brain. The unique capacity for complementation of soluble lysosomal proteins to be secreted by cells and taken up at a distance by others ('secretion–recapture') renders those lysosomal diseases in which neurological manifestations are prominent as excellent targets for clinical exploration of gene therapy (see Chapter 12.8). Here, the principle of allowing a proportion of neural cells to be stably trans- duced by vector, thus to serve as a source of a given corrective pro- tein that can be taken up into the lysosomes that lack the enzyme in nearby neurons, is an attractive strategy for therapeutic exploration. Although there are some prospects of correcting some enzyme defects in the somatic cell genome, the correction of defects in the germline seems remote, although the development of advanced in vitro fertilization techniques, preimplantation DNA analysis, gene transfer, insertion or conversion, and embryo implantation pro- cedures may render this possible. However, the prospect of human germline modification will arouse many complex ethical issues, and these may hold up research and clinical application. Screening for inborn errors of metabolism The realization that very early diagnosis is essential in order to achieve good results in the treatment of many inborn errors of metabolism, such as phenylketonuria and galactosaemia, has stimulated interest in the possibility of examining either whole populations or selected groups of predisposed individuals for the biochemical differences which characterize particular inherited metabolic diseases. Diagnosis is needed at a stage which is not only presymptomatic but which pre- cedes the onset of self-perpetuating secondary pathological changes. Screening for inborn errors of metabolism may be either non- selective (whole population) or selective. The latter, which includes carrier detection studies, aims to cover a part of the population. This may be defined on clinical, genetic, ethnic, or geographical grounds. Phenylketonuria and congenital hypothyroidism are the only metabolic disorders for which neonatal whole-population screening is generally practised, although medium chain fatty acid dehydro-genase deficiency has been included recently in the United Kingdom and many other countries. There is wide international variation and galactosaemia, cystic fibrosis, and congenital adrenal hyper- plasia (21-hydroxylase deficiency) have been proposed. Until recently it has been held that whole-population screening should only be established for treatable or preventable diseases, and the con- sistency of the association of the proposed biochemical or other marker and the serious clinical phenotype must have been proved beyond any doubt. There must be a reliable and robust analytical method suitable for use with a sample of blood or urine which can be obtained without distressing either the parents or the baby. The possibility that metabolic screening will bring to

light previously unrecognized variants, which are either mild and do not require treatment, or which by virtue of a fundamentally different bio- chemical lesion will resist the currently established therapies, has to be borne in mind. Phenylketonuria illustrates these difficulties. Here, beside classical phenylketonuria, whole-population screening has identified both the clinically unimportant essential (mild) hyperphenylalaninaemia, and the devastatingly serious, but treat- able, inborn errors of tetrahydrobiopterin synthesis which produce the 'malignant' hyperphenylalaninaemia syndrome. In a subset of patients with classical phenylketonuria, tetrahydrobiopterin also

 improves blood phenylalanine control, and may, in the long term, allow the burden of stringent dietary treatment to be relaxed. It is also possible that in some cases immediate postnatal screening and treatment may be too late to prevent minor manifestations of the disease (e.g. in congenital hypothyroidism). The incidence of disease which merits whole-population screening should be at least similar to that of phenylketonuria in white Europeans (between 1 in 6000 and 1 in 14 000). Cystic fibrosis has a birth frequency of approximately 1 in 2500 (heterozygous car- rier frequency l in 25) in white persons of European ancestry and would merit neonatal whole-population screening on this basis. Molecular genetic approaches are potentially useful. If the dis- ease is not too genetically heterogeneous, and when the full range of possible causative mutations is known, the specific mutation could be sought directly. Some individuals classified as being homozy- gotes on the basis of classical genetic analysis prove to be compound ('double') heterozygotes, that is, they carry two different mutations, each affecting either the maternal or paternal allele of the same gene. The number of inborn metabolic errors in which the affected indi- viduals and the heterozygous carriers can be identified by molecular analysis of genomic DNA is increasing rapidly. The conditions which are identified by the application of DNA analytical methods include such numerically important diseases as sickle cell anaemia, β-thalassaemia, haemophilia, Duchenne muscular dystrophy, cystic fibrosis, medium-chain acyl-CoA dehydrogenase deficiency, and phenylketonuria, as well as rarer but devastating conditions such as the Lesch–Nyhan syndrome. Prenatal diagnosis The procedures used in prenatal diagnosis are: • direct examination of the fetus by ultrasonography and fetoscopy • chemical analysis of amniotic fluid • biochemical and cytological analysis of cultured amniotic cells (amniocytes) obtained by amniocentesis at weeks 15 to 16 of pregnancy • DNA analysis on uncultured amniocytes • karyotypic enzymological and DNA analysis of chorionic villi obtained by biopsy at weeks 10 to 12 of pregnancy • biochemical studies on tissue obtained by fetal biopsy in utero • sequencing of circulating cell-free DNA (cfDNA) during pregnancy. Carrier state diagnosis Carriers are either individuals carrying the gene for a recessive dis- order, which does not express itself in the heterozygous state (e.g. phenylketonuria), or those who carry the gene for a dominant dis- order, that is, one which does express itself in the heterozygous state, but in which symptoms occur in later life (e.g. Huntington's disease). The general approaches to carrier state diagnosis are: • detection of minor clinical, radiological, and clinicopathological abnormalities • demonstration of levels of enzyme activity in tissue (e.g. leuco- cytes or cultured fibroblasts) which are intermediate between those observed in individuals homozygous for the abnormal and the normal forms of the enzyme respectively (the observed level of activity may not be exactly 50% of the normal value) • demonstration of intermediate levels of a characteristic metabolite in an accessible body fluid • demonstration of mosaicism with respect to the product of the mutant gene on the X chromosome in the case of sex-linked reces- sive disorders • direct gene analysis using either a specific gene probe or a linked restriction fragment length polymorphism. The ability

to recognize asymptomatic carriers of serious recessive diseases and presymptomatic individuals in the case of dominant disorders raises major ethical and social issues with respect to the psychological impact that this information will have on the affected individuals and their families. This is especially so with the clinically normal carriers of a crippling, lethal, and untreatable disease such as Huntington's disease. Applications of genome sequencing Recent years have seen astonishing improvements in the accuracy, extent, and rate at which genetic information can be accessed by sequencing of human DNA. In the four decades since robust methods for determining the nucleotide base order in natural DNA molecules were first reported, the growth of sequence information has been astronomical as a result of 'next-generation sequencing' methods. Meanwhile, the desire to generate and store these data has also burgeoned. The simultaneous explosion in bioinformatics and statistical genetics combined with the need for clinical prognostication is driving a revolution. Analysis dependent on massive parallel sequencing has intensified knowledge of the pathological anatomy of the human genome; research is ongoing to conduct simulations and molecular modelling and incorporate empirical structural biology to realize a key scientific ambition with the aim of improving the capacity to predict the clinical consequences of genomic variants. DNA sequencing now extends far beyond the domain of laboratory science and evolutionary studies: in biology it has proliferated cat- egorically and dominantly for clinical use. Rapid, cheap, and increas- ingly reliable, DNA sequencing applications in medicine are enabling predictive testing in certain circumstances, for example, highly pene- trant Mendelian disorders caused by recurrent variants, but this is the exception rather than the rule for most genetic disorders. Now DNA analysis is beginning to support natural appetites for clinical prognostication: for diagnosis, decision-making, therapeutic discovery, and drug targeting, but knowledge of the clinical implications of genomic variation is currently very incomplete, as emphasized by the fact that, of approximately 20 000 genes in the human genome, only about 30% have a known role in human disease. Each human genome contains 4 to 5 million variants (positions where the genetic code in the indi- vidual differs from the human reference sequence), and distinguishing disease-causing variants from rare background variants can be very challenging. We are only beginning to learn how to integrate DNA sequencing information into clinical practice, and there can be con- siderable dangers in assuming that a particular DNA sequence has (or will have) a particular clinical impact in a particular patient. In clinical contexts where there is limited experience of sequencing methodology, there needs to be caution in the diagnostic field and it has been suggested that the following stratagems be adopted: (1) obtain detailed clinical phenotyping to create a carefully thought out and justifiable differential diagnosis—if it seems biologically unlikely that a particular gene is involved in the generation of a particular

 phenotype, then it is likely to be wrong to assume that a mutation in that gene is causal; (2) scrutinize the family history and mode of inheritance, and compare (when possible) gene sequence in the proband with that in other family members; and (3) confirm DNA sequence data by manual inspection of the sequencing reads for genes that are of interest, and check these by independent methods approved for clinical use. It is also appropriate to point out that mas- sive parallel sequencing does not detect all genetic abnormalities: it may be necessary to deploy alternative sequencing such as Sanger sequencing and deletion/duplication testing to detect single nucleo- tide and copy number deletion and in-frame deletions/duplications that can readily be missed. In the field of rare diseases, of which the huge catalogue of inborn errors of metabolism occupies a central place, DNA sequencing is of arresting interest for practitioners. The perennial need for prompt diagnosis carries with it the

prospect of comfort and utility when faced with diagnostic challenges and countless choice, and also has potential implications for mass screening as much as for the stricken individual. Sequencing circulating cell-free DNA in pregnancy The most widespread clinical application of DNA testing is pre- natal testing for fetal chromosomal abnormalities, such as trisomy 21 in Down's syndrome. As of 2017, up to 6 million women have undergone sequence analysis of cfDNA for fetal aneuploidy (see Chapter 3.9). cfDNA is obtained at about 10 weeks of pregnancy from small samples of maternal blood. Described accurately as the 'fastest growing genetic test in medical history', this technology, introduced as a plasma analyte after 20 years of research by Dennis Lo and col- leagues, offers great hope for applications of DNA sequencing in clin- ical medicine—for example, in cancer diagnosis and monitoring and for diagnosis and screening in single-gene disorders. In relation to screening for inborn errors and rare diseases gener- ally, 'liquid' biopsy of fetal DNA circulating in maternal blood offers potentially easy access to the genomic DNA of the fetus at risk in a noninvasive manner. However, while testing for fetal aneuploidy and some cancers has little need for nucleotide-level accuracy (chromo- somes can be counted without checking for sequence variation), the application to noninvasive diagnosis of suspected genetic diseases as well as de novo screening for single-gene disorders has more fas- tidious requirements. Given the potential value and need for such testing, however, rapid progress has been made: there are reliable means to detect fetal rhesus D genotypes in rhesus D-negative women, and some skeletal dysplasias can be identified when the ultrasonographic findings prompt suspicion. Most inborn errors of metabolism are autosomal recessive dis- orders and here it is first necessary to quantify the abundance of maternal alleles or haplotypes in the fetus relative to the wild-type counterpart in the maternal DNA component. Relative dosages of maternal DNA and fetal in plasma have been used successfully to predict transmission of sickle cell anaemia, ß-thalassaemia and haemophilia- care being taken to obtain sequence from fetal cfDNA components to assemble the structure of the haplotype flanking the putative mutant locus. At the time of writing, inborn errors such as congenital adrenal hyperplasia due to 21-hydroxlase de- ficiency and the X-linked lysosomal disease, Hunter syndrome (mucopolysaccharidosis type II), have been successfully diagnosed by analysis of cfDNA in maternal plasma. In vitro fertilization and the inborn errors of metabolism The human embryo produced by in vitro fertilization can be biopsied at a very early stage of development (i.e. at the eight-cell stage). A single cell is removed and examined for the DNA mutation responsible for the disease which the parents are known to be carrying or for parental haplotypes tightly linked to the parental mutations). This technique, known as preimplantation genetic diagnosis, enables only embryos which do not carry the disease-causing genotype to be implanted. Animal genetic models of inborn errors of metabolism in humans Animal models of the inborn errors of metabolism occur spontan- eously and have been used in therapeutic research for many years, but the capacity to generate models of genetic disease by transgenic techniques has greatly advanced this avenue of exploration. Not only do such models offer the hope of shedding important light on the mechanisms of disease, they have much to offer in the develop- ment of innovative treatments before attempting to transfer these to patients—now referred to as translational medical research. The discovery of embryonic stem cells in the mouse and the ability to manipulate the mammalian genome by targeted homologous recom- bination have been instrumental in generating 'knock-out' models of human genetic diseases. Once the cognate nuclear gene of the mouse has been disrupted in embryonic stem cells, these cells are injected into the inner cell mass of individual host blastocysts. In some of the resultant chimeric embryos, the embryonic stem cells harbouring the mutant locus contribute to the development of the gonads in the adult progeny; ultimately, when this is the case, offspring can be bred to

homozygosity for the disrupted locus and studied. Refinements of this technology based on the use of regulatory sequences and tissue-specific promoter elements permit the target locus to be manipulated at will in the whole animal at a predefined stage of development by the administration of small molecules that bind to control elements (inducible ‘knock-out’ and/or ‘knock-in’ models models) or allow the genetic locus of interest to be deleted in particular tissues (conditional knock-out model). Murine and other living experimental models of human diseases are valuable in medicinal research but limitations to the method- ology remain when cognitive and behavioural abnormalities are critical features of the clinical phenotype in patients; even with the constraints of recruitment in individually rare diseases, the experi- mental system by which innovative treatments are best tested for use in human patients remains the clinical trial. The future Astonishing progress has been made in the understanding, diag- nosis, and treatment of inborn errors of metabolism and it is clear that the future holds immense promise for continued advancement and introduction of credible therapies for conditions that until re- cently were beyond hope—either for clinical control, rescue, or even reversal. Prodigious research efforts to accelerate and perfect understanding of many rare inborn disorders are now bearing fruit. Molecular genetics, cell biology, and biochemistry have been pro- ductively collated into clinical practice as a result of a contemporary explosion of knowledge about the genome, the associated lexicon of

 inherited disease, and the derived information about protein struc- ture and function. For these reasons, it seems that the future highlights of this rare and formerly neglected clinical field will be dominated by spin-out discoveries that ultimately relate to the study of DNA. Genetic sequence data Incremental improvements in the application of genomic sequen- cing will be used routinely to investigate infants and children with undiagnosed conditions, especially those in whom a strong gen- etic basis is suspected. Increasingly in private health facilities and services available in resource-rich societies, this technology will be extended to older patients. Depending on the analytical strata- gems chosen and whether whole-exome or genomic sequencing is adopted, even now some providers claim that disease-causing muta- tions are identified in up to one-third of patients. It is almost inevitable that economies of scale resulting from cen- tralized national provision, and refinements in the bioinformatic interrogation and hierarchical stratagems used to analyse the data, will enhance its value for earlier diagnosis, and (one hopes) better coordinated access to counselling and specialized care. With ex- perience and improved information about human genetic variation in different populations, greater clarity will emerge on the clinical value of this approach. With introduction of large population-scale studies such as the Biobank resource in the United Kingdom and the All of Us research programme in the United States of America, data interpretation and family studies will become better aligned to the experience and prac- tices of local diagnostic teams. In many cases, the validated diag- noses obtained by next-generation sequencing lift the health of the patient and ultimately enhance clinical care with appropriate service provision. Greater experience with these methods will overcome the numerous pitfalls of whole-exome and whole genome sequencing, where overzealous betrayal of time-honoured clinical principles can lead to diagnostic errors and chaotic management. Careful clinical testing and the importance of phenotype-guided molecular testing to drive diagnosis, together with confirmation of diagnosis by in- dependent methods (e.g. biochemical analysis), should, with time, minimize these disturbing errors due to lack of experience and expertise. Liquid biopsy and maternal plasma cfDNA sequencing will in- creasingly translate to clinical care of genetic diseases. As the discov- erers of this technology are aware, the application of more precise screening represents a critical advance in medicine, with reduced need for invasive

procedures will attract significant policy debate as inevitable ethical challenges present themselves. Specific therapies The benefit of specific therapies that target specific molecular defects in which cause-and-effect pathogenesis has been established is an area of intense aspiration and, now, productive endeavour for fu- ture application. The interventions already include augmenting gene therapies (e.g. recombinant adeno-associated viral vectors systems that transduce nonmitotic cells and third-generation integrating lentiviral vectors that transduce actively dividing cells) and gene suppression therapies that diminish RNA expression and thus re- duce biosynthesis of harmful mutant or unregulated wild-type pro- tein (e.g. antisense methods and use of small interfering RNAs) have also recently been approved for clinical use (transthyretin-related amyloidosis and polyneuropathy). Other molecular therapies offering generalizable principles for expanded application include the protein channel potentiator drugs for class III mutations of the cystic fibrosis transmembrane regulator protein, CFTR. Mutation-specific stratification of CFTR therapy with the singe agent, ivacaftor, facilitates increased chloride trans- port by potentiating the channel-open probability (or gating) of the G551D-CFTR variant protein. Progress in access to orphan therapies With a burgeoning interest in so-called personalized medicine, in part driven by the pharmaceutical industry, beyond distinct cancer variants, inborn errors of metabolism are increasingly seen as the exemplary case for therapeutic exploration. The advantages of the orphan drug legislation (including 7–10 years of marketing exclu- sivity in the United States of America and Europe, respectively) pro- vide strong incentives for the development of innovative agents for such rare diseases with identified genetic targets. On an individual basis, many orphan agents are exceptionally costly for each patient and thus have attracted considerable political attention for reim- bursement. More challenging will be the commercial development of treatments involving one-off interventions such as gene therapy, because here the ‘cost models’ allowing credible investment by bio- pharmaceutical organizations and shareholders have yet to be put in place. While there is not space here to discuss these matters (see ‘Further reading’), they will undoubtedly come to increasing polit- ical importance as new and effective medicines are introduced for inborn errors of metabolism and cognate rare disorders. FURTHER READING Accurso FJ, et al. (2010). Effect of VX-770 in persons with cystic fibrosis and the G551D-CFTR mutation. N Engl J Med, 363, 1991–2003. Alison MR, Islam S, Lim SM (2009). Cell therapy for liver disease. Curr Opin Mol Ther, 11, 364–74. Altshuler D, Daly MJ, Lander ES (2008). Genetic mapping in human disease. Science, 322, 881–8. Auricchio A, Smith AJ, Ali RR (2017). The future looks brighter after 25 years of retinal gene therapy. Hum Gene Ther, 28, 982–7. Bainbridge JWB, et al. (2008). Effect of gene therapy on visual function in Leber’s congenital amaurosis. N Engl J Med, 358, 2231–9. Barry PJ, Donaldson AL, Jones AM (2018). Ivacaftor for cystic fibrosis. BMJ, 361, 287–9. Bearn AG (1993). Archibald Garrod and the individuality of man. Oxford University Press, Oxford. Bianchi DW, Chiu RWK (2018). Sequencing of circulating cell-free DNA during pregnancy. N Engl J Med, 379, 464–73. Biffi A (2017). Hematopoietic gene therapies for metabolic and neuro- logic diseases. Hematol Oncol Clin North Am, 31, 869–81. Buckley B (2008). Clinical trials of orphan medicines. Lancet, 371, 2051–5. Cartier N, et al. (2009). Haemopoietic stem cell therapy with a lentiviral vector in X-linked leukodystrophy. Science, 326, 818–23. Childs B (2004). A logic of disease. In Scriver CR, et al. (eds) Metabolic and molecular bases of inherited disease, 8th edition. McGraw-Hill, New York. http://www.ommbid.com. Chinnery PF (2015). Mitochondrial disease in adults: what’s old and what’s new? EMBO Mol Med, 7, 1503–12.

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