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ESSENTIALS Lysosomal function and classification of diseases The lysosome is a ubiquitous, single membrane-bound intracellular organelle which continuously recycles biological macromolecules: it not only breaks down cell components but has a dynamic role in nu- trient and energy sensing that, through regulatory signalling, is critical for homeostasis and metabolic economy of the cell. More than 80 lysosomal diseases caused by single gene defects are known. These are classified according to the nature of the pri- mary storage molecules (biochemical classification) or according to the defective molecular cell physiology (functional classification), or (more usefully) a combination of these classification systems that in- corporates genetic information. Biochemical classification identifies (1) sphingolipidoses; (2) mucopolysaccharidoses; (3) glycoproteinoses; (4) glycogenosis, with or without lysosomal debris derived from subcellular organ- elles due to impaired autophagy; and (5) miscellaneous condi- tions with multiple classes of storage material such as the neuronal ceroid lipofuscinoses. Functional classification describes deficiency of (1) a specific acid hydrolase activity, (2) an activator protein,
(3) a lysosomal membrane protein or transporter, or (4) abnormal post-translational modification of lysosomal proteins, and (5) ab- normal biogenesis of lysosomes. A unified classification will emerge from genetic characterization integrated with clinicopathological manifestations of the individual disorders. Clinical features and diagnosis About one in 5000 live-born infants have a lysosomal disorder. Clinically diverse, the lysosomal diseases can appear at any age but are very rarely present at birth. Diagnosis is usually suspected on the basis of key clinical presentations of progressive neurodegenerative disease, often combined with visceral enlargement (especially splenomegaly), connective tissue and skeletal disease, or particular syndromic appearances. As with all disorders with strong hereditary determinants, diagnosis is crucial for prognosis and genetic counselling, because specific ther- apies may have disease-modifying effects, and to prevent inappro- priate interventions (e.g. removal of an enlarged spleen). A detailed family history, including careful analysis of the extended pedigree, is essential. Diagnosis is confirmed by biochemical methods including examination of urine metabolites or specific enzymatic assays on leucocytes or cultured fibroblasts, histochemical stains of existing bi- opsy material, and/or next-generation DNA sequencing studies. Particular lysosomal diseases Fabry’s and Gaucher’s diseases

(glycosphingolipidoses) are prob- ably the most frequent in the general population, but certain lyso- somal diseases are over-represented in particular groups where consanguinity or endogamy is high (e.g. the high frequency of non-neuronopathic Gaucher's disease, infantile and late-onset Tay– Sachs disease (GM2 gangliosidosis), and Niemann–Pick disease type A (neuronopathic) in Ashkenazi Jews). Fabry's disease—an X-linked disorder caused by deficiency of α-galactosidase A, which leads to the accumulation of globo triaosylceramide (Gb3), typically manifests in early childhood with lancinating pain and background burning sensations in the extrem- ities. Other features include diarrhoea, lack of peripheral sweating, impotence, high-tone deafness, angiokeratomas, chronic kidney disease, hypertrophic cardiomyopathy, and stroke. Enzyme therapy with recombinant human α-galactosidase A is very costly but im- proves neuropathic pain and cardiac hypertrophy. Gaucher's disease is an autosomal recessive trait caused by func- tional deficiency of acid glucocerebrosidase. Characteristic manifest- ations of the most frequent form—'adult non-neuronopathic' (type 1)—include pancytopenia, splenic enlargement, and bone pain with osteoporosis and episodic osteonecrosis. Parenteral administration of enzyme therapy with imiglucerase (Cerezyme), velaglucerase alfa (VPRIV), taliglucerase alfa (Elelyso) or oral substrate reduction therapy with miglustat (Zavesca) or eliglustat (Cerdelga) is extremely expensive but clinically effective. Other diseases discussed in this chapter include (1) cystinosis, (2) the mucopolysaccharidoses, (3) Pompe's disease (glycogen storage disease type II), (4) Niemann–Pick diseases, (5) lysosomal acid lipase deficiency, (6) Danon's disease, and (7) diseases more recently attributed to primary defects in lysosomes and related organelles. Treatments Despite some striking therapeutic advances in several lysosomal conditions such as Gaucher's disease, there is no specific or curative 12.8 Lysosomal disease Patrick B. Deegan and Timothy M. Cox

section 12 Metabolic disorders 2122 treatment for most lysosomal disorders. Supportive and palliative measures are nonetheless of great benefit. Advanced cell and molecular therapies can have striking benefits in several lysosomal diseases: these include bone marrow (haem- atopoietic stem cell) transplantation, specific augmentation with receptor-targeted recombinant human lysosomal enzymes, sub- strate biosynthesis inhibitors, pharmacological chaperones, and sub- strate dissolution stratagems. Gene therapy is in a promising phase of clinical development for this group of disorders. Lysosomal function Since their discovery more than 65 years ago by the late Nobel prize winner Christian de Duve, lysosomes and their associated endosomal structures have been at the centre of research into mo- lecular cell biology and membrane dynamics. Lysosomes are an in- tegral part of the intracellular digestive system (see Chapter 3.1) and acquire complex macromolecules for breakdown and recycling by three main pathways: (1) receptor-mediated endocytosis, (2) en- gulfment and fusion (phagocytosis), and (3) autophagy. Greater understanding of lysosomal function has arisen from biochemical definition of cellular macromolecules that accumulate when the or- ganelle is affected by hereditary diseases. Many other molecular components that bring about trafficking and membrane-fusion events, for example, Rab proteins and SNARE proteins, are localized to, and interact with, the lysosome. These proteins form complexes that bring about the ebb and flow of substrates and digestive products as they move between com- partments in the greater lysosomal network within the cell. Live- cell imaging techniques reveal a highly dynamic constellation of particles that are continuously moving and fusing as a result of the release of phosphate-bond energy through the action of GTPases. This chemical bond energy is used to drive the continuous traf- ficking of membrane constituents involved in the innumerable tran- sient interactions of the endosomal, lysosomal, and

autophagosome compartments. Endocytosis and membrane flow Receptor-mediated endocytosis occurs by means of clathrin-coated pits, a process by which molecules are delivered after internaliza- tion to a peripheral, and later to a perinuclear endosomal compart- ment, ‘the endolysosome’. The endolysosome undergoes maturation to form a lysosome after the loss of certain membrane components and further acidification. Some molecules acquired by receptor-mediated endocytosis (e.g. apolipoprotein B in low-density lipoproteins) are specifically retrieved and returned ultimately to the cell surface, having des- patched their cargo to the lysosome. Other molecules that are not retrieved are ultimately degraded by fusion with mature lysosomes and enzymatic hydrolysis (e.g. the epidermal growth factor receptor system). The endosomal system also mediates the traffic of nascent acid hydrolases from the trans-Golgi network to the lysosome, employing a specific mannose 6-phosphate receptor targeting system. Plasma membrane proteins bound for degradation in the lysosomal system are incorporated into membrane-bound vesicles within the endosomal lumen and thus enter the lysosome upon fusion. In contrast, structural or transporter proteins bound for incorporation into the lysosomal membrane remain within the limiting membrane of the endosome and form part of the lyso- somal membrane on fusion. Two multicomponent macromolecular complexes, CORVET (core subunit vacuolar/endosome tethering complex) and HOPS (homotypic fusion and protein sorting), bring about vacuolar fu- sion with the endosomal, lysosomal, and autophagosome com- partments. Recently, a few patients with destabilizing mutations in HOPS components have been investigated. While the cell biology is incompletely understood, these patients will be instructive since the complex phenotype of Turkic patients with mutations in the vacuolar protein-sorting associated protein, VP33A, clearly has pathological lysosomal storage with features resembling a mucopolysaccharidosis (MPS) despite a full complement of lyso- somal acid hydrolases. Further investigations of such extraordinary patients are likely to reveal much about the functional dynamics of lysosome–endosome pathways in cell biology and their relevance to medicine. Phagocytosis Lysosomes are also involved in a specialized process for the degrad- ation of exogenous particulates and proteins, including microbes and effete cells such as erythrocytes and neutrophils. Although this engulfment and fusion process involving phagolysosomes is a feature of many cells, it is particularly active in macrophages and dendritic cells. In macrophages, cell surface components on bacteria and yeast, as well as exogenous cells, are recognized and bound by specific receptors on the plasma membrane. The phagocytes engulf for- eign material to form large vesicles in which acidification and proteolysis, as well as the secretion of degradative molecules (including reactive oxygen and nitrogen species), is initiated. The phagolysosome fuses with lysosomes and further acidification oc- curs, so that the acid hydrolases are activated to effect destruction of the ingested material. A specialized phagolysosome variant occurs in osteoclasts that are derived from myeloid cells of mononuclear phagocyte origin. The osteoclastic resorptive vacuole serves as a large exteriorized lyso- somal compartment which is independently acidified for resorption of bone. Autophagy Autophagy occurs within cells: microautophagy describes the degradation of cytosolic components trapped during invagin- ation of endosomes and lysosomes, while macroautophagy de- scribes the engulfment of relatively large volumes, including organelles. In a constant process of membrane fusion and flow, the endo- plasmic reticulum (reticulophagy), ribosomes, mitochondria (mitophagy), peroxisomes and other lysosomes, and particulate ma- terial such as macromolecular complexes of glycogen are engulfed by autophagic vacuoles. Formation of these vacuoles is initiated when a flattened cisterna composed of membrane, the phagophore, encir- cles cytoplasm to form a double-layered vesicle, the autophagosome: acidified late endosomes and lysosomes fuse with the nascent vacu- oles to form an autolysosome.

12.8 Lysosomal disease 2123 With progressive acidification, the complement of lysosomal hydrolases effects breakdown of the inner membrane and digestion of the vacuolar contents. After digestion is completed, the autolysosome acquires an electron-dense—and often autofluorescent—core known as a residual body. When lysosomal function is impeded, the breakdown of endogenous macromolecules is impaired; this, together with a failure to break down exogenous macromolecular substrates, results in a characteristic pattern of pathological storage of the biological residue. Disturbed autophagy is a spectacular fea- ture of cystinosis, Pompe's disease, and Danon's disease, but prob- ably drives much of the pathogenesis of many, if not all, lysosomal diseases, including those involving cells where autophagy is particu- larly active, as in neurons. Recent exploration of the central signalling pathway that controls tissue and cell growth, the mechanistic target of rapamycin com- plex 1 (mTORC1), has shown that under nutrient-rich conditions this complex is recruited to the cytoplasmic surface of the lyso- some where it interacts with a large assembly of proteins including a small guanine triphosphatase, Rheb, which is itself regulated by the prevailing energy charge, oxygen availability, and growth factor signalling. Under conditions in which amino acids are not abun- dant, mTORC1 dissociates from the membrane and is inactive. The energy-requiring acidifying molecule, vacuolar (V)-ATPase, which pumps protons into the lysosome, is also part of the assembly and may be influenced by amino acid abundance. The condition of X- linked myopathy with excess autophagy illustrates the role of the nutrient-sensing feedback loop that controls autophagy; defects in a chaperone, VMA21, that assembles the V-ATPase give rise to inadequate lysosomal acidification and reduced release of nutrient amino acids, thus leading to upregulated autophagic processes. Autophagy retrieves the basic building blocks of cellular compo- nents and proceeds hand-in-hand with de novo synthesis and the re-newal of intracellular compartments throughout life; as summarized earlier, the process is stimulated under conditions of starvation and disuse—for example, in immobilized muscles or during involution of the anterior pituitary or mammary gland after pregnancy and lac- tation. When starvation is prolonged, macroautophagy slows down in favour of the lysosomal uptake of a class of large cellular proteins harbouring particular amino acid sequences which are recognized by receptors that mediate import into the organelle. The intrinsic and highly glycosylated lysosomal membrane protein, LAMP2, is implicated in the uptake of such cytosolic proteins. LAMP2 is mu-tated in the X-linked disorder Danon's disease in which liver as well as cardiac and skeletal muscle show prominent vacuoles engorged with glycogen and other debris, including remnants of cellular organelles. Common regulation of autophagy, lysosomal biogenesis, and lysosomal function A common regulatory system based on a transcription factor, TFEB, upregulates many of the processes required for integrated function of autophagy: formation of vesicles, production of factors necessary for cargo recognition and fusion of the autophagosome and lyso- some, synthesis of lysosomal enzymes, membrane proteins, and pH pumps. Phosphorylation of TFEB prevents its entry into the nucleus and hence its actions on the transcription of genes harbouring the responsive binding sequences in the CLEAR element. The system is activated by dephosphorylation of TFEB, indirectly after inhib- ition of the master-regulator mTOR, a kinase that is controlled in part by the effects of low cytoplasmic concentrations of leu- cine and arginine as well as low-energy charge during periods of starvation. Deficiency of the activity of one lysosomal enzyme usually leads to a coordinated upregulation of many proteins including those en- coded by genes containing CLEAR sequences that coordinate lyso- somal function and autophagy. Degradation and recycling of complex macromolecules Of the 400 or so proteins found in the lysosome, including membrane proteins, there are at least 50 lysosomal hydrolases. These include proteases, glycosidases, sulphatases, phosphatases, nucleases, lipases, and phospholipases. These enzymes

require an acidic pH for optimal function, which in the lysosome is maintained at a pH of 4 to 5.5 by the ATP-dependent proton transporter V-ATPase. The lysosomal mem- brane and the acidic pH optimum of the hydrolases protect other cell components, at neutral pH, from indiscriminate autodigestion. Lysosomes display a range of pH values, properties that are linked to their position in the living cell (e.g. perinuclear or peripheral). Live-cell microscopic imaging reveals highly dynamic structures that move rapidly in three dimensions with oscillating acidities that may reflect functional transients which change according to the flux of different substrates. Intralysosomal pH values could influence the rate of substrate hydrolysis according to the differential pH op- timum of particular reactions and thus the exchange of macromol- ecular substrates between distinct organelle populations during the digestive sequence. Targeting of hydrolases About 60 known soluble lysosomal enzymes and activator proteins continually digest and recycle cellular macromolecules. Most of these components are targeted to the lysosome by the mannose 6-phosphate cotranslational pathway. Immediately after biosynthesis, N-linked oligosaccharides on lysosomal proteins are modified spe- cifically to generate the principal mannose 6-phosphate recognition marker with the addition of a phosphate group on the sixth carbon of mannose residues. The appropriately phosphorylated proteins bind to mannose 6-phosphate receptors in the trans-Golgi network and the ligand–receptor complexes enter clathrin-coated transport vesicles that traffic to an acidic prelysosomal compartment where the ligands dissociate. Soluble lysosomal proteins enter the lyso- somal matrix and most of the receptors recycle back to the trans- Golgi network to repeat the intracellular delivery process. About 20% of the mannose 6-phosphate receptors traffic to the cell surface, where their complement of protein ligands is released but where fresh binding and hence recapture is stochastically possible. This creates the potential for glycoproteins harbouring the appro- priate hexosyl phosphate recognition signal that are discharged into the fluid phase to be distributed and recaptured by cell surface man- nose 6-phosphate receptors. While this process may have a physio- logical role, for example, in regulating insulin-like growth factor (IGF) concentrations in plasma, because the cation-independent M6P receptor also functions as the IGF2 receptor its greatest signifi- cance is to provide the foundation for functional complementation of lysosomal diseases due to deficiency of soluble proteins (in this example, iduronate sulphatase).

section 12 Metabolic disorders 2124 Activator proteins Activator proteins are required for several lysosomal en- zymes: saposin C is required for the in vivo catalytic function of glucocerebrosidase, deficiency of which causes Gaucher’s disease. Indeed, a Gaucher’s disease-like phenotype has been observed in the rare individuals with saposin C deficiency. The small protein, GM2 activator, is absolutely required for the digestion of GM2 ganglioside by ß-hexosaminidase A and deficiency of the GM2 activator causes a disease with clinical features similar to Tay- Sachs disease. Another vivid example of a crucial activator for many lysosomal enzymes is sulphatase-modifying factor 1 (SUMF1), an enzyme ex- pressed in the endoplasmic reticulum that catalyses the oxidation of a conserved cysteine residue within a protein domain of all known sulphatases, many of which are involved in the breakdown of glyco- saminoglycans and sulphated glycosphingolipids in the lysosome. Homozygous or compound heterozygous mutations in the human SUMF1 gene lead to multiple sulphatase deficiency (Austin’s disease) with features of MPS and metachromatic leukodystrophy as well as very dry skin, ichthyosis, due to defective activity of cutaneous steroid sulphatase. Transporter proteins Transporter proteins of the lysosomal membrane are less well characterized than the acid hydrolases. Maintaining a proton gra- dient with a concentration of protons approximately 1000-fold greater than that in the ambient cytosolic pH depends on the

pres- ence of a V-type H+ ATPase in the lysosomal membrane. Other membrane transporters exist for the crucial counter-exchange of ions, including the chloride channel, CLCN7. This channel is expressed in the endosome–lysosomal membranes of all cells but is abundant on the ruffled border of the osteoclast. Mutations in the CLCN7 gene are responsible for dominantly inherited and se- vere autosomal recessive forms of osteopetrosis due to a failure to acidify the subosteoclastic vacuole, an externalized lysosomal as- sembly of enzymes critical for modelling bone matrix during ossification and maintenance of skeletal integrity throughout life. In the recessive variants, mutations of CLCN7 are also responsible for neurodegeneration. Other channels include those that transport Ca2+ (LAAT1), as well as the egress of small molecules generated by hydrolytic digestion of the macromolecular substrates in the lysosome— for example, cystinosin (cystine), CblF (cobalamin), and sialin (sialic acid) which are implicated respectively in cystinosis, methylmalonic acidaemia due to defective intracellular vitamin B12 transport, and Salla disease. Transporters are required to export the products of lysosomal digestion, such as amino acids, monosaccharides, nucleosides, and ions, for reuse in cellular me- tabolism. Recent research has identified mucolipin as a channel for monovalent cations; impaired function of which causes mucolipidosis IV. The protein CLN3, deficiency of which results in a juvenile type of neuronal ceroid lipofuscinosis type 3 (‘Batten’s disease’), has been implicated in the egress of arginine. Antigen presentation Proteases of lysosomal origin, particularly the cysteine proteinases or cathepsins, are responsible for the cleavage of endocytosed protein antigens to generate peptide fragments. In antigen-presenting cells, where abundant expression of several of these proteases is necessary, peptide fragments of the cognate antigens are presented in associ- ation with major histocompatibility complex (MHC) class II mol- ecules as a key step in the pathway that orchestrates the adaptive immune response. In mouse models of autoimmunity, deficiency of acid proteases—such as cathepsins B and S, generated experimen- tally by gene disruption technology—can ameliorate many of the disease manifestations. Definition and classification of lysosomal diseases Definition Lysosomal diseases result from inherited defects in lysosomal hydrolases and the mechanisms for delivering them to the organelle, lysosomal enzyme activators and cofactors, lysosomal membrane proteins, and carrier systems for the transport of the substrates and products of lysosomal digestion between the organelle and the cyto- plasm. Most of the enzymatic defects are restricted to the activity of a single hydrolase, but defects of activators and cofactors, as well as proteins involved in the processing of nascent lysosomal enzymes for organellar delivery, can lead to generalized defects of lysosome function. Classification Lysosomal disorders have been classified according to the nature of the primary storage compounds (biochemical classification) or ac- cording to the nature of the physiological defect (functional classifi- cation). A combination of two classification systems, incorporating genetic characterization, may allow a clearer description of the pathological basis of the condition. As the clinical manifestations of the 80 or more diseases associ- ated with inborn errors of lysosomal function are very diverse, the reader is referred to specialized literature for further information (see ‘Further reading’). Biochemical classification Sphingolipidoses Sphingolipids are amphiphilic compounds with a lipophilic moiety based on the amino-alcohol sphingosine (usually linked to a long-chain fatty acid to form ceramide) and a polar hydrophilic mono- or oligosaccharide chain; in sphingomyelins, which are the most abundant sphingolipids, the charged head group is either phosphorylcholine or phosphorylethanolamine. Sphingolipids are found in all plasma membranes and concentrated in large aggre- gates: the lipophilic moiety is often anchored by structural proteins such as tubulin in the lipid bilayer and the carbohydrate element or head group extends into the extracellular space. Sphingolipids mediate diverse cellular functions and serve as spe- cific receptors and cell recognition markers. They are continuously

delivered to the lysosomal compartment in the course of mem- brane turnover in endocytosis, phagocytosis, and by autophagy. Deacylated forms of the sphingolipids (β-d-glucosylsphingosine, a ‘psychosine’, is the deacylated form of glucosylceramide), are water-soluble and hence diffusible. Exploration of the roles of

12.8 Lysosomal disease 2125 such lysolipids and other lipid molecules, such as sphingosine 1-phosphate and ceramide, in signal transduction and other cellular processes is an expanding field of research and holds promise for a more comprehensive understanding of the molecular pathogenesis of sphingolipid diseases. Mucopolysaccharidoses Mucopolysaccharides, or glycosaminoglycans, are complex linear polysaccharides, composed of repeating units of polar disaccharides which are strongly negatively charged under physiological condi- tions; glycosaminoglycans contain amino sugars substituents and are often sulphated. When associated with a linear core protein, the glycosaminoglycans form even larger three-dimensional complexes, known as proteoglycans. By virtue of their negative charge and extended structure, proteo-glycans attract water molecules and have important gel-like proper- ties. Proteoglycans are essential components of ground substance in intercellular spaces and connective tissue, including cartilage, vit- reous humour, and synovial fluid. Lysosomal degradation of the carbohydrate moieties requires the participation of several glycosidases orchestrated in series. Lysosomal diseases associated with the failure to digest heparan sul- phate in particular, such as Hunter’s disease and Sanfilippo’s diseases (MPS II and MPS III subtypes), are all associated with prominent neurological disease. Glycoproteinoses Glycoproteins are proteins to which one or more oligosaccharide chains are attached covalently. The carbohydrate moiety is often branched and complex, mediating specific recognition by cell sur- face receptors. As with the glycosaminoglycans, lysosomal degrad- ation of the carbohydrate element requires the ordered participation of several glycosidases operating in sequence. Deficiency of one glycosidase, in effect, blocks the subsequent release of sugars in the reaction series, thus causing accumulation of oligosaccharides and other complex glycan molecules. Glycogenosis (Pompe’s disease) Deficiency of α1,4-glucosidase, acid maltase, causes intra- and extralysosomal accumulation of glycogen in muscle and other tis- sues. This storage molecule is common to conditions of impaired autophagy (e.g. Danon’s disease) but is prominent in Pompe’s disease and confirms the role of the lysosome in constitutive and continuous remodelling of glycogen macromolecules. Multiple classes of storage material Several lysosomal hydrolases are not specific to one substrate: β- galactosidase deficiency, for example, leads to accumulation of a glycosphingolipid (GM1 ganglioside) and glycosaminoglycans (keratan sulphate and β-galactosyl oligosaccharides) with greatly divergent clinical manifestations. This enzyme deficiency is respon- sible for a range of phenotypes, extending from a predominantly neurodegenerative disease, GM1 gangliosidosis, to the skeletal dis- order of Morquio B (MPS IVB) in which neurological impairment is typically absent. All eukaryotic sulphatases, including the eight sulphatases destined for the mammalian lysosome—where they catalyse the removal of sulphate moieties from glycosaminoglycans, glycolipids, and glycopeptides—require the conversion of a conserved cysteine residue to formylglycine for their activation. Genetic defects in the enzyme, sulphatase-modifying factor (SUMF1), which mediates this conversion in the endoplasmic reticulum, leads to Austin’s dis- ease (multiple sulphatase deficiency)—a condition which usually resembles late-infantile metachromatic leukodystrophy but with prominent clinical and biochemical features of a complex MPS as well as ichthyosis, due to an accompanying deficiency of steroid sulphatase. Deficiency in the precursor of the small sphingolipid acti- vator proteins leads to loss of activity of the cognate hydrolases, whose

activity depends on interaction with the activators saposins A to D. Depending on the selectivity of the defect, a distinct dis- ease complex, characterized by accumulation of a broad panel of glycosphingolipids, occurs when these proteins are deficient. A genetically and biochemically distinct activator, GM2 acti- vator protein, interacts crucially to form a ternary complex with the two subunits of hexosaminidase A and its specific natural sub- strate in the lysosome, GM2 ganglioside. Deficiency of this activator protein gives rise to a phenocopy of the severe neurodegenerative disorder Tay–Sachs disease, but characteristically the activity of hexosaminidase A, when determined with the usual fluorogenic substrates, is unimpaired. A similar phenomenon occurs in the dis- orders due to saposin deficiencies and may render correct diagnosis difficult for the unwary. Most acid hydrolases are glycoproteins that are specifically tar- geted to the lysosomal system through interaction between a mannose-phosphate moiety and membrane mannose-phosphate receptors. Failure to generate the mannose-phosphate signal gives rise to widespread mistargeting of hydrolases and intralysosomal deficiency of the respective activities with consequent accumu- lation of many substrates (I-cell disease); characteristically, body fluids, including plasma, have increased activities of many lysosomal hydrolases and although the disease shares many features of a MPS, the urine is usually free of glycosaminoglycans. Miscellaneous Please see Table 12.8.1 for details. Functional classification Classification based on the nature of the defect in cell biological terms is particularly relevant in relation to potential therapeutic ap- proaches. Examples of the type of defects listed here are given in the 'Biochemical classification' section, in Table 12.8.1, and throughout the text: • Deficiency of a specific acid hydrolase activity • Deficiency of an activator protein • Deficiency of a lysosomal membrane protein or transporter • Abnormal post-translational modification of lysosomal proteins • Abnormal lysosomal biogenesis Deficiency of an acid hydrolase may come about either as a result of a mutation that reduces catalytic activity, or as a result of a muta- tion that impedes correct folding of the protein and delivery of the mutant enzyme to the lysosome. This has implications for pathogen- esis and potential therapeutic approaches.

section 12 Metabolic disorders 2126 Table 12.8.1 Representative classification of lysosomal diseases Disease Synonym OMIM Locus, gene Gene product and functional classification Storage material Sphingolipidoses Farber Lipogranulomatosis 228000 8p22 ASAH Acid ceramidase Acid hydrolase Cer Fabry Anderson–Fabry 301500 Xq22 GLA α-Galactosidase A Acid hydrolase Gb3 Gaucher Glucosylceramidosis 606463 230900 231000 230800 1q21 GBA Glucocerebrosidase Acid hydrolase GlcCer GM1 gangliosidosis 230500 230600 3p21 GLB1 β-Galactosidase Acid hydrolase GM1 Tay–Sachs GM2-gangliosidosis B 272800 15q23 HEXA β-Hexosaminidase α-subunit Acid hydrolase GM2 Sandhoff GM2-gangliosidosis O 268800 5q13 HEXB β-Hexosaminidase β-subunit Acid hydrolase GM2 Tay–Sachs AB variant GM2 gangliosidosis AB 272750 5q32 GM2A GM2 activator protein Activator protein GM2 Krabbe Globoid cell leukodystrophy 245200 14q31 GALC β-Galactosylceramidase Acid hydrolase GalCer Metachromatic leukodystrophy Arylsulphatase A deficiency 250100 22q13 ARSA Arylsulphatase A Acid hydrolase Sulphatide Prosaposin deficiency 176801 10q22 PSAP Prosaposin Activator protein Multiple lipids Saposin B deficiency Metachromatic leukodystrophy variant 249900 10q22 PSAP Saposin B Activator protein Sulphatide Saposin C deficiency Gaucher variant 610539 10q22 PSAP Saposin C Activator protein GlcCer Niemann–Pick types A and B 257200 607616 11p15 SPMPD1 Acid sphingomyelinase Acid hydrolase SM Other lipidoses Niemann–Pick type C1 257220 18q11 NPC1 NPC1 Probable transmembrane transporter Cholesterol, GSL Niemann–Pick type C2 607625 14q24 NPC2 NPC2 Soluble transporter Cholesterol, GSL Wolman Cholesteryl ester storage disease 278000 10q23.2 LIPA Acid lipase Acid

hydrolase Cholesteryl esters Mucopolysaccharidoses (MPS) MPS I Hurler Hurler/Scheie, Scheie 607015 (MPS IH) 607015 (MPS IHS) 607016 (MPS IS) 4p16 IDUA α-Iduronidase Acid hydrolase DS, HS MPS II Hunter 309900 Xq28 IDS Iduronate sulphatase Acid hydrolase DS, HS MPS IIIA Sanfilippo A 52900 17q25 SGS Heparan N-sulphatase Acid hydrolase HS MPS IIIB Sanfilippo B 252910 17q21 NAGLU N-acetyl glucosaminidase Acid hydrolase HS MPS IIIC Sanfilippo C 252930 8p11 TMEM76 HGSNAT α-Glucosaminide acetyl-CoA transferase Acid hydrolase HS MPS IIID Sanfilippo D 252940 12q14 GNS N-acetylglucosamine 6-sulphatase Acid hydrolase HS MPS IVA Morquio A 253000 16q24 GALNS Galactosamine 6-sulphatase Acid hydrolase KS,CS MPS IVB Morquio B 253010 3p21 GLB1 Acid β-galactosidase Acid hydrolase KS

12.8 Lysosomal disease 2127 Disease Synonym OMIM Locus, gene Gene product and functional classification Storage material MPS VI Maroteaux–Lamy 253200 5q12 ARSB Arylsulphatase B N-Acetyl galactosamine 4-sulphatase Acid hydrolase DS MPS VII Sly 253220 7q21 GUSB Glucuronidase Acid hydrolase DS, HS, CS MPS IX Hyaluronidase deficiency 601492 3p21 HYL1 Hyaluronidase 1 Acid hydrolase HA Glycogen storage disease Pompe Glycogen storage disease type II 232300 17q25 GAA α-Glucosidase Acid hydrolase Glycogen Multiple substrate storage due to single gene defects Multiple sulphatase deficiency Austin disease 272200 3p26 SUMF1 Formyl-glycine generating enzyme Post-translational modification Sulphatide, mucopolysaccharides Galactosialidosis 256540 20q13 PPCA Protective protein Cathepsin A Acid hydrolase GSL, polysaccharides Mucolipidosis type II I-cell disease 252500 12q23 GNPTAB UDP-GlcNac Phospho transferase α/β unit Post-translational modification Multiple lipids and oligosaccharides Mucolipidosis type IIIa Classic pseudo-Hurler's polydystrophy 252600 12q23 GNPTAB UDP-G1cNac Phosphotransferase α/β unit Post-translational modification Multiple lipids and oligosaccharides Mucolipidosis type IIIb Pseudo-Hurler's polydystrophy 352605 16p GNPTG UDP-G1cNac Phosphotransferase γ-subunit Post-translational modification Multiple lipids and oligosaccharides Mucolipidosis type IV 252650 19p13 MCOLN1 Mucolipin-1 cation channel Transporter Multiple lipids and oligosaccharides VP33A deficiency Mucopolysaccharidosis plus syndrome Turks and Yakut 610034 12q24.31 VP33A is a common component of the heterohexameric tethering complexes involved in endocytic-lysosomal fusion: HOPS (homotypic fusion and vacuole protein sorting) and the Rab GTPase-dependent CORVET (class C core vacuole/ endosome tethering) Glycosaminoglycans, especially heparan sulphate; increased acidification Glycoproteinoses Aspartylglucosaminuria 208400 4q32 AGA Glycosyl-asparaginase Acid hydrolase Aspartyl-glucosamine Fucosidosis 230000 1p34 FUCA α-Fucosidase Acid hydrolase Oligosaccharides α-Mannosidosis 248500 19q12 MAN2B1 α-Mannosidase Acid hydrolase Oligosaccharides β-Mannosidosis 248510 4q22 MANBA β-Mannosidase Acid hydrolase Oligosaccharides Sialidosis Sialidase deficiency Mucolipidosis type 1 256550 6p21 NEU1 α-Sialidase (neuraminidase) Acid hydrolase Oligosaccharides Schindler NAGA deficiency Kanzaki's disease 609242 609241 22q13 NAGA α-N-acetyl galactosaminidase Acid hydrolase Oligosaccharides Lysosomal transport defects Cystinosis Abderhalden–Kaufmann– Lignac disease 219800 219900 219750 17p13 CTNS Cystinosin, a transmembrane protein Cystine efflux Transporter Cystine Methylmalonic aciduria Vitamin B12 lysosomal release defect 277380 Unknown Vitamin B12 carrier (Cb1F) Transporter Vitamin B12 Table 12.8.1 Continued (continued)

section 12 Metabolic disorders 2128 Disease Synonym OMIM Locus, gene Gene product and functional classification Storage material Salla Sialuria 604322 6q14 SLC17A5 Sialin Transporter Sialic acid Lysosomal protease defect Pycnodysostosis 265800 1q21 CTSK Cathepsin K Acid

hydrolase Collagen fibrils (osteoclasts) Autophagy defects (with glycogenosis) Danon Pseudoglycogenosis II 300257 Xq24 LAMP2 LAMP2 Membrane protein Vacuoles, macromolecular debris, glycogen, and membrane RNASET2 Leukoencephalopathy, cystic without megalencephaly; RNase T2-deficient leukoencephalopathy 612951 6q27 Ribonuclease T2 deficiency (glycoprotein with endoribonuclease activity and acid optimum) Unknown in humans: but implicated in reticulophagy (endoplasmic reticulum autophagy)—authentic zebrafish model shows accumulated rRNA in neurons X-linked myopathy with excessive autophagy 310440 Xq28 XMEA VMA21 Membrane protein Vacuole Vacuolar myopathy Muscular dystrophy with vacuoles 601846 19p13 MDRV MDRV Unknown function Vacuoles Autophagy defects X-linked vacuolar myopathy with excessive autophagy (XMEA) MEAX 310440 Xq28 VMA 21, chaperone for the lysosomal V-ATPase. Vacuoles Neuronal ceroid lipofuscinosis (NCL) CLN1 (infantile, late- infantile, juvenile, adults) Haltia–Santavuori 256730 1p32 CLN1/PPT1 PPT1 palmitoyl protein thioesterase 1 Acid hydrolase SAPs (sphingolipid activator proteins) CLN2 (late infantile/juvenile) Jansky–Bielchowsky 204500 11p15 CLN2/TPP1 TPP1 tripeptidyl peptidase 1 Acid hydrolase SCMAS (subunit c mitochondrial ATP synthase) CLN3 (juvenile) Spielmeyer–Sjögren Batten 204200 16p12 CLN3 CLN3 (Battenin) transmembrane protein— endosomes, lysosomes as well as synaptic vesicles SCMAS CLN4A (adult) Kufs type A—adult (autosomal recessive) 204300 15q23 CLN6 CLN6 Membrane transporter SCMAS CLN4B Kufs, Parry disease (autosomal dominant) 162350 20q13.33 (DNAJC5) Soluble, cytoplasmic cysteine string protein alpha— palmitoylation reduced SAPs CLN5 Finnish (late infantile, juvenile, adult) 256731 13q22.3 CLN5 CLN5—a complex but soluble glycoprotein SCMAS CLN6 Lake–Cavanagh Late infantile, adult—Kufs type A variant 601780 15q23 CLN6 CLN6 Transmembrane protein possible ER transporter SCMAS CLN7 vLINCL (Turkish and others) 610951 4q28.2 MSFD8 CLN7/MFSD8 Transmembrane protein SCMAS CLN8 Northern epilepsy 600143 8p23 CLN8 CLN8 Transmembrane protein transfers lysosomal enzymes from Golgi to endoplasmic reticulum SCMAS [CLN9] Obsolete Original Serbian-German pedigree reclassified as CLN5 after genetic studies 609055 (obsolete) N/A N/A N/A CLN10 Congenital with microcephaly, neonatal and late infantile CLN (very rare juvenile and adult variants) 610127 11p15 CTSD Cathepsin D Acid hydrolase SAPs Table 12.8.1 Continued

12.8 Lysosomal disease 2129 Disease Synonym OMIM Locus, gene Gene product and functional classification Storage material CLN11 Autosomal recessive (note: heterozygotes with GRN mutations develop autosomal dominant frontotemporal dementia with TDP43-inclusions— OMIM 607485) 614706 17q21.31 GRN Progranulin Intraneuronal ubiquitin-positive autofluorescent lipofuscin and rectilinear inclusions on electron microscopy CLN12 Juvenile (note: recessive mutations in ATP13A2 also cause Kufor–Rakeb syndrome, PARK9, juvenile- onset atypical Parkinson’s disease with supranuclear gaze palsy, spasticity and dementia (OMIM 606693)) 610513 1p36.13 CLN12/ATP13A2 ATPase type 13A2 Lysosomal membrane protein CLN13 Adult CLN (sometimes known as Kufs B—autosomal recessive) 615362 11q13.2 CTSF Cathepsin F Soluble acid hydrolase Autofluorescent material CLN14 Infantile—progressive myoclonic epilepsy 611725 7q11.21 CLN14 (KCTD7) Potassium channel tetramerization domain containing protein 7—a putative cytoplasmic regulator of potassium conductance Fingerprint or rectilinear as well as osmiophilic deposits by electron microscopy Disorders in extended lysosomal apparatus (melanosomes, lamellar bodies) Chédiak–Higashi 214500 1q42 LYST LYST Biogenesis defect Enlarged vacuoles Melanosomes MYOV Griscelli type 1 214450 15q21 MYO5A Myosin 5A Biogenesis defect Melanin granules RAB27A Griscelli type 2 603868 15q21 RAB27A RAB27A Biogenesis defect Melanin granules Melanophilin Griscelli type 3 609227 2q37 MLPH Melanophilin Biogenesis defect

Melanin granules HPS-1 Hermansky–Pudlak type 1 604982 10q23 HPS1 HPS-1 Biogenesis defect Multiple vacuoles HPS-2 Hermansky–Pudlak type 2 608233 5q14 AP3B1 AP3 β-subunit Biogenesis defect Multiple vacuoles HPS-3 Hermansky–Pudlak type 3 606118 3q24 HPS3 HPS-3 Biogenesis defect Multiple vacuoles HPS-4 Hermansky–Pudlak type 4 606682 22q11 HPS4 HPS-4 Biogenesis defect Multiple vacuoles HPS-5 Hermansky–Pudlak type 5 607521 11p15 HPS5 HPS-5 Biogenesis defect Multiple vacuoles Biogenesis defect Multiple vacuoles HPS-6 Hermansky–Pudlak type 6 607522 10q24 HPS6 HPS 6 Biogenesis defect HPS-7 Hermansky–Pudlak type 7 607145 6p22 DTNB1 Dysbindin Biogenesis defect Multiple vacuoles HPS-8 Hermansky–Pudlak type 8 609762 19q13 BLOC1S3 BLOC1S3 Biogenesis defect Multiple vacuoles HPS-9 Hermansky–Pudlak type 9 614171 15q1.21 BLOC1S6 Pallidin Biogenesis defect HPS-10 Hermansky–Pudlak type 10 617050 19p3.3 AP3D1 Biogenesis defect Surfactant metabolism dysfunction-4 SMPD3 610921 16p13 ABCA3 ABCA3 transporter Alveolar proteins Congenital and lamellar ichthyosis Harlequin fetus 242500 601277 2q34 ABCA12 ABCA12 transporter Abnormal keratin Cer, ceramide; CS, chondroitin sulphate; DS, dermatan sulphate; Gb3, globotriaosylceramide; GlcCer, glucosylceramide; HA, hyaluronan; HS, heparan sulphate; KS, keratan sulphate; SM, sphingomyelin. Table 12.8.1 Continued

 Pathophysiology Lysosomal storage of primary substrates In the initial period of discovery of lysosomal diseases, limited avail- ability of investigative tools influenced how the conditions were viewed. Pathological examination and microscopy showed not only organ enlargement but intracellular ‘storage’ bodies of remarkable appearance. Biochemical characterization of storage compounds guided research into discovery of enzyme activities and gene prod- ucts. This led to a somewhat simplistic view of the pathogenesis of lysosomal storage diseases as being related to the expansion of cells and organs containing relatively inert ‘storage’ material. In most cases, the contribution of storage material to organ enlargement is quantitatively very small and the widespread effects of lysosomal diseases on cell–cell interactions with paracrine, inflammatory, and immunological consequences, remain unsolved. Latterly, the patho- genesis of lysosomal diseases in relation to so-called secondary metabolites and their specific roles in signalling and cell biology is receiving attention; investigations are also underway into the effects of storage on membrane flow and trafficking within the cell. Although the amount of storage material that accumulates within lysosomes in the lysosomal diseases may be several hundred- or thousand-fold greater than normal, the absolute amount of material may amount to only a few grams, even in an enlarged viscus such as the spleen, which may exceed 5 kg in some disorders. For example, the presence of a few grams of the sphingolipid, sphingomyelin, in Niemann–Pick disease types A and B, is associated with massive vis- ceral enlargement with accompanying inflammatory, ischaemic, and other destructive changes due to the presence of storage cells. Similarly, marked pathological injury occurs: in the viscera and bone marrow spaces of patients with Gaucher’s disease; in the heart and skeletal muscles of patients with α-glucosidase deficiency (with glycogen ac- cumulation in the sarcoplasm of striated and cardiac myocytes); in the kidney and heart of patients with Fabry’s disease; and affecting neurons throughout the nervous system of patients with ceroid neur- onal lipofuscinosis, Tay–Sachs disease, and GM1 gangliosidosis. Secondary metabolites and their effects Although there often appears to be an anatomical relationship be- tween the extent of lysosomal storage and the development of overt disease in a particular organ, at present there is little mechanistic understanding of this relationship in molecular terms. Sphingolipids participate in cell recognition events and receptor biology; sphingo- lipid metabolites (the deacylated lysosphingolipids) also function as signalling molecules in

apoptotic and proliferative responses. However, in two striking instances (Gaucher's and Krabbe's dis- eases, due to acid β-glucocerebrosidase and β-galactocerebrosidase deficiencies, respectively), multinucleated macrophages resem- bling the pathognomonic Gaucher's or globoid cell can be induced in vitro by the water-soluble molecules glucosylsphingosine and galactosylsphingosine (psychosine), which are overproduced in these diseases. At pathophysiological concentrations in culture, psychosines and related glycolipids inhibit cytokinesis and for this reason are im- plicated in the molecular pathways responsible for the presence of large macrophage-like cells with several nuclei in the brain of patients with Krabbe's disease ('globoid cells') and in the marrow, spleen, and other viscera in Gaucher's disease ('Gaucher cells'). Psychosines (glucosylsphingosine and galactosylsphingosine) ap- pear to interact with receptors on the plasma membrane of human monocytic-lineage cells. Latterly, multiple myeloma, which occurs at a greatly increased frequency in Gaucher's disease, has been reported to result from a T-follicular helper type 2 (TFH2)-mediated B-cell prolif- erative response directed against the pathological glycosphingolipids present in serum (especially ß-glucosylsphingosine), with clonal para- protein antibody reactivity directed against this circulating lipid mol- ecule in patients with Gaucher's disease. Up to one-third of patients with spontaneous monoclonal gammopathy apparently unrelated to Gaucher's disease have been reported to have specific antibody reactivity to ß-glucosylsphingosine or other sphingolipids such as lysophosphorylcholine. Another deacylated glycosphingolipid, lyso-globotriaosylceramide (lyso-GB3), has been identified in the plasma of patients with Fabry's disease. This induces smooth muscle proliferation and is thus impli- cated in the severe systemic and cerebrovascular manifestations that characterize the condition. These biochemical findings may signify new approaches to the understanding of several lysosomal disorders associated with cell loss due to apoptosis and fibroinflammatory responses; since lysolipids diffuse readily from their site of formation, different ap- proaches to their treatment other than targeted enzyme replacement may be appropriate. Cellular effects The cellular reaction associated with lysosomal storage is often re- stricted and stereotypical. In neural tissue, several pathological hall- marks such as meganeurite formation and ectopic dendritogenesis accompany the accumulation of a wide assortment of storage com- pounds. It appears that lysosomal storage gives rise to a general- ized defect in the complex traffic flow mediated by the endosomal system with effects on autophagy, signal propagation at the synapse, axonal transport, myelin formation, and arborization of dendrites. Lipid rafts, detergent-resistant islands within the plasma membrane, contain high local concentrations of gangliosides and play an im- portant role in many cell signalling events. Disordered lateral move- ment and recycling of raft components as part of a general or specific endosomal 'traffic jam' may have profound effects on cell signalling, as well as recycling processes mediated by autophagy. As an example of such cellular cascades, Niemann–Pick C1 dis- ease has been linked to a sequence of events in which sphingosine accumulation causes depletion of acidic compartment calcium stores, leading to accumulation of various lysosomal lipids including cholesterol. Disorders in which autophagy is markedly disturbed, particularly those affecting the nervous system, can lead to mito- chondrial dysfunction as a result of impaired clearance of effete and damaged organelles (defective mitophagy). The causative link between Gaucher's disease and Parkinson's disease remains to be established, but one area of active research implicates the overproduction of mutant protein which then fails to fold correctly, accumulating within the endoplasmic reticulum, activating the unfolded protein response, the final effects of which may include apoptosis. Individuals who are heterozygous for pathological GBA1 mutations not only develop parkinsonian fea- tures but also Lewy body dementia: in such patients, the Lewy bodies costain for α-synuclein, the mutant ß-glucosylceramidase and ubiquitin.

12.8 Lysosomal disease 2131 Tissue and organ malfunction In a scientific era that offers powerful analytical techniques to ex- plore complex functional networks which lead to tissue pathology, the lysosomal diseases represent a promising field for investiga- tion using large-scale, high-throughput methods to investigate al- tered protein and gene expression in the context of cell signalling responses. An early application of this work has been the use of authentic experimental models of some of the more severe storage diseases generated by gene knockout technology, which facilitate re- search on otherwise inaccessible tissues such as the brain during the development of the storage phenotype. Gene expression profiling experiments conducted during periods of neuronal cell death have shown upregulation of genes related to the inflammatory process in the nervous system of mice that serve as a model of GM2 gangliosidosis. The activation of local microglia is shown by the signature of upregulated macrophage expression markers and lymphocyte chemoattractants, as well as genes encoding antigen-presenting MHC class II molecules. Since Krabbe's disease modelled in mice is mitigated by bone marrow transplantation, which supplies a population of genetically competent immune cells (and which is accompanied by the use of powerful immunosuppres- sion), it seems probable that the altered immunity accompanying bone marrow transplantation may itself modify the clinical expres- sion of lysosomal diseases affecting the brain, and such an effect may be independent of the storage material. Several indirect studies have indicated the release of inflamma- tory cytokines in at least one lysosomal storage disease (Gaucher's disease), which may explain the metabolic and plasma protein ab- normalities associated with a sustained inflammatory response that characterizes the clinical syndrome. Hypertrophy and fibrosis char- acterize the organ responses in many lysosomal diseases. Whether the response is mediated at a cellular, paracrine, or endocrine level remains unclear. In Fabry's disease, lyso-Gb3 is a promising candi- date for an elusive, endocrine-like factor in the cardiac and vascular aspects of this condition. The clinical presentation of malfunctioning organs in lysosomal storage disorders generally resembles the pathological outcomes of hypertrophy, fibrosis, and organ failure observed in other chronic conditions. Neurological syndromes vary with the anatomical site of greatest injury and with the relative involvement of grey or white matter (neuronopathic or myelination defects). Clinical presentation Natural course and severity range All lysosomal diseases disturb the catabolism of complex molecules in numerous tissues and their manifestations are usually progres- sive and permanent. They show no relationship to food intake and are generally independent of intercurrent illness. The rate of de-terioration depends in part on the degree of residual activity of the deficient enzyme or process: subtotal deficiencies present early in childhood with rapid evolution of disease; partial deficiencies emerge more slowly and often present in later childhood or adult life. The disease may be insidious, as in the indolent splenomegaly of adults with Gaucher's disease, the renal impairment of Fabry's dis- ease, or the muscle weakness of adult-onset Pompe's disease. Acute episodes may punctuate this process, giving rise to a step-wise im- pairment of function, such as occurs with the osteonecrosis that typically affects the epiphyses of the long bones in Niemann–Pick disease type B or Gaucher's disease. Organomegaly and disturbed visceral function Those disorders that affect metabolically active organs such as the liver and kidney often cause functional impairment, including the manifestations of liver failure, portal hypertension, and—in the case of the kidney—rickets and metabolic acidosis, for example, as a con- sequence of Fanconi's syndrome in cystinosis. Cardiac involvement leads to hypertrophy, diastolic dysfunction, conduction and rhythm disturbances, as well as thickening of the valves. Respiratory mani-festations of the mucopolysaccharidoses are usually first evident as a result of narrowing of large airways, but restricted ventilation due to skeletal disease often supervenes. Splenomegaly,

complicated by marked functional hypersplenism, is characteristic of untreated Gaucher's disease. Skeletal manifestations Skeletal effects predominate and are particularly cruel in several of the mucopolysaccharidoses. Severe growth retardation, joint stiffness, and atlantoaxial instability impair the quality and dur- ation of life. In Gaucher's disease, diverse osseous manifestations include marrow infiltration, osteoporosis, osteonecrosis, lytic lesions, pathological fractures, and occasional plasmacytoma or frank myelomatosis (Fig. 12.8.1). Neurological features Lysosomal diseases are a prominent cause of progressive neuro- logical and mental deterioration in patients whose disease starts during adolescence up to mature adult life, and they should always be considered in the differential diagnosis of such presentations. Ataxia is a feature of GM1 and GM2 gangliosidoses, and a flaccid paraparesis in young children might suggest meta- chromatic leukodystrophy. Widespread white-matter disease in association with frontal dementia and spastic paraparesis is a char- acteristic presentation of juvenile and adult forms of metachrom- atic leukodystrophy and Krabbe's disease. Polyneuropathy and pyramidal signs are superimposed in both disorders. Early-onset leukodystrophy is caused by metachromatic leukodystrophy, mul- tiple sulphatase deficiency, and Krabbe's disease. The latter is a rare but important diagnostic entity in this group since the disease may be partially ameliorated by allogeneic marrow transplantation in very early life. Lysosomal diseases with prominent neurological manifestations are often associated with progressive mental deterioration, with or without the onset of spasticity, myoclonic seizures, and optic atrophy. Extrapyramidal signs including parkinsonism, athetoid movements, and dystonia are also frequent. Corneal opacities suggest cystinosis, I-cell disease, mucopoly saccharidoses, mannosidosis, Fabry's disease, and galactosialidosis, as well as one form of Gaucher's disease with neuronopathic features (the D409H type IIIc variant). Perifoveal pallor with the appearance of pigmentation in the macula (the 'cherry-red spot' in white per- sons) is a hallmark of Tay–Sachs disease and other gangliosidoses affecting infants and young adults. Specific syndromes are described in later sections.

section 12 Metabolic disorders 2132 Diagnosis Clinical suspicion and family history Even in the critically ill, it is essential (whenever possible) to estab- lish the definitive diagnosis where a lysosomal disease is suspected, for several reasons: (1) specific treatment may be possible—enzyme replacement therapy, bone marrow transplantation, or even oral substrate reduction and enzyme enhancement therapies may be available; (2) these disorders are inherited either as X-linked or as autosomal recessive traits, which has important consequences for reproductive choice in other family members; and (3) the diagnosis may clarify unexplained symptoms in at-risk relatives. The key to making the diagnosis of a rare lysosomal disease is often informed suspicion combined with dogged persistence. In most circumstances, once suspected, the lysosomal disease can be identified with relative ease by referral to a specialized regional ref- erence laboratory for the diagnosis of metabolic disorders: senior laboratory staff will usually advise about the handling of appro- priate tissue material for diagnostic studies, including the means for securing a genetic diagnostic by molecular analysis of genomic DNA (see 'Molecular diagnosis'). In the first instance, simple histochemical stains of existing biopsy material and examination of urine metabolites, including lipids and oligosaccharides, may narrow down the diagnosis. More commonly, specific enzymatic assays are used. These are generally carried out on leucocytes isolated from fresh heparinized blood samples, or on fibroblasts cultured from small biopsy specimens of skin; the latter are particularly valuable since, once established, fibroblast cultures can be stored indefinitely for repeated and definitive study. Fabry's disease, Niemann–Pick disease types B and C, as well as Gaucher's disease have often come to light in young or adult

patients with particular syndromic presentations. Apart from paediatricians, general physicians, haematologists, nephrologists, neurologists, gastroenterologists and hepatologists, dermatologists, and even orthopaedic surgeons may be the first to evaluate the patient—all of whom should be able to identify the condition by following diag- nostic pathways appropriate to their specialty. In any event, the diag- nosis of lysosomal storage diseases is rarely difficult, provided the expertise of trusted laboratory services is available for the conduct of biochemical assays, diagnostic DNA studies, and wide-ranging histopathological examination. The value of good communication between laboratory staff and clinical investigators, to whom these patients are referred, cannot be overestimated. Pattern of inheritance A detailed family history, taking care to investigate the extended pedigree, is of critical importance for these high-penetrance mono- genic diseases. Most lysosomal diseases are inherited as autosomal recessive traits and patients have biallelic mutations at the locus pri- marily implicated. The differential diagnosis narrows substantially if there is evidence of X-linked inheritance characteristic of Fabry's disease, MPS II (Hunter's disease), or Danon's disease. At-risk family members will be identified, often living with presymptomatic or undiagnosed disease. Tracing family members is best carried out sensitively in cooperation with professional genetic counselling services and with the general oversight of patient representative organizations. Particular populations While the possibility of frank consanguinity should be explored (usu- ally from pairings between cousins), it is helpful to be aware of rare diseases that are over-represented in small populations with high endogamy. Examples include the high frequency of Hermansky– Pudlak syndromes 1 and 3 among Puerto Ricans and certain isolated Swiss communities. Lysosomal diseases are also more frequent than (a) (b) Fig. 12.8.1 (a) A 35-year-old male with α-mannosidosis. (b) A 20-year-old male with α- mannosidosis and coincident glutaric aciduria type 1. Facial appearance includes prominent brow, depressed nasal bridge, and prognathism. Note hearing aids.

 expected in Ashkenazi Jews, including Hermansky–Pudlak type 3, Niemann–Pick disease (type A), and type 1 (non-neuronopathic) Gaucher's disease. Type 3 (chronic neuronopathic) Gaucher's dis- ease is frequent in parts of arctic Sweden and in one caste group in Pakistan, among which several hundred patients have been identified. The high frequency of several mutant alleles of the HEXA gene re- sponsible for Tay–Sachs disease in Ashkenazi Jews has led to greatly enhanced awareness of the disorder in this population, with suc- cessful international programmes for carrier detection. The birth of infants soon to be affected by Tay–Sachs disease is now exceptional in the Ashkenazim, but infantile as well as attenuated, late-onset forms of the disease occur with a high frequency in isolated popu- lations such as Moroccan Jews, French settlers in Quebec (Canada), the Cajun people of Louisiana (United States of America), and the Canadian Metis Indian population. Sandhoff's disease, due to mutations in the HEXB gene and the other major form of GM2 gangliosidosis, is not over-represented in Jews but occurs at high frequency in the aboriginal population in the district of Cordoba (Argentina) and in the Arabic followers of the Syriac Maronite Church in Lebanon, Syria, and Northern Cyprus. Infantile Krabbe's disease occurs with a very high carrier fre- quency among the Druze people of Northern Israel and Lebanon and appears to be more frequent in Scandinavian countries and other parts of Northern Europe but an adult variant arising from a single missense mutation in the GALC gene is occurs at high fre- quency Catania, Italy. Radiology Ultrasonography, MRI, and CT may reveal visceral enlargement and infiltration, for example Niemann–Pick disease, mucopoly saccharidoses, and Gaucher's disease. Skeletal radiographs may reveal bone expansion in vertebrae and in the phalangeal and long bones, sometimes associated with infarction and collapse, par- ticularly in

Niemann–Pick disease type B and Gaucher's disease. Echocardiography may reveal thickening and calcification of the cardiac valves (particularly of the aortic ring), infiltration of cardiac muscle causing ventricular hypertrophy in Pompe's disease, Fabry's disease, mucopolysaccharidoses I, IV, and VI, and, often strikingly, in Danon's disease. Neuroradiology is informative, particularly in patients with mucopolysaccharidoses and in Morquio's syndrome as well as MPS syndromes I, II, and VI where instability of the atlantoaxial joint may cause fatal subluxation as a consequence of connective tissue disease abutting the dens. MRI of the cervical spine in MPS is critical for assessing when to carry out posterior fusion to stabilize the joint. Similarly, investigations of the lower spine may determine the cause of progressive spinal deformity due to lumbar kyphosis and assist in the evaluation of the need for surgery. MRI of the brain is invaluable in the assessment of dementing illnesses: cortical and/or white matter disease may be delineated. Imaging plays a key part in diagnosis of the striking white matter changes of Krabbe's disease, multiple sulphatase deficiency, and metachromatic leukodystrophy (Fig. 12.8.2). Extensive white matter lesions and eventual cerebral atrophy also characterize the advanced stage of the neurological aspects of Fabry's disease (Fig. 12.8.3). Pathology Although lysosomal defects occur in all tissues, the principal focus of each disease is manifest in those tissues with the most rapid turn- over of the parent macromolecule of which degradation is impaired. For example, in Gaucher's disease the turnover of parent glycolipids appears to be greatest in the mononuclear phagocytes. Here the ac- cumulation of glycolipids derived from the breakdown of complex sphingolipids present in white cell and red cell membranes present in the formed blood elements occurs. With mild or moderate im- pairment of the responsible enzyme, glucocerebrosidase (acid ß- glucosylceramidase), the pathology is restricted principally to the macrophage-containing tissues of the liver, spleen, bone marrow, and (occasionally) the lung. When inherited defects further impair the activity of the enzyme, the nervous system becomes a site of dis- ease: here the main source of accumulating glucosylceramide and glucosylsphingosine is derived from the recycling of the endogenous cellular sphingolipids, particularly gangliosides present in neuronal membranes. Microscopic pathology shows storage within dilated vesicular spaces, which represent diseased lysosomes. Sphingolipids, being amphipathic molecules, tend to accumulate in whorls known as 'membranous cytoplasmic bodies' where they assume a lamellar structure within lysosomal spaces. Paracrystalline and crystal- line material in distended lysosomes may also be seen under elec- tron microscopy, for example, in the accumulation of the charged glycolipid, sulphatide, that occurs in metachromatic leukodystrophy Fig. 12.8.2 T2-weighted MRI of the brain of a young woman with adult-onset metachromatic leukodystrophy—psycho-cognitive variant. Notice the high signal intensity, especially in the frontal white matter and periventricular regions. This patient presented with bizarre behaviour due to a frontal-type dementia; there were no neurological signs or symptoms. Near total loss of short-term memory with lack of planning and higher executive functions were prominent features of her illness.

 (arylsulphatase A deficiency). With more water-soluble substrates, granular material accumulates within the vesicular spaces. These spaces represent distended and often fused lysosomes, filled, for ex- ample, with undegraded glycogen macromolecular complexes in acid maltase deficiency (Pompe's disease). As emphasized earlier, the pathological manifestations of the lysosomal diseases are diverse. They may range from enlargement of viscera with infiltration by abnormal macrophages containing storage material (foam cells of Niemann–Pick disease or Gaucher cells) to bone infarction, neuronophagia, vacuolation of renal tubular cells, and diverse tissue infiltrates. Inclusion bodies may be observed

in metachromatic-stained cells of the urine deposit or in circulating neutrophils and lymphocytes (Maroteaux–Lamy disease, MPS VI); staining with a periodic acid–Schiff reagent may reveal diastase-resistant glycolipid storage in the kidney and other organs in Fabry's disease and other glycosphingolipidoses. The presence of metachromatic storage material in nervous tissue, including periph- eral nerves, is characteristic of the sphingolipidosis, metachromatic leukodystrophy (Fig. 12.8.4). The secondary effects of lysosomal ex- pansion related to upregulation of lysosomal proteins through the TFEB/CLEAR transcriptional pathway include increased staining for tartrate-resistant (type 5) acid phosphatase, hexosamindases and other lysosomal markers, and intrinsic membrane proteins, for example, LAMP1. Ultrastructural examination is often diagnostic for lysosomal dis- eases: membrane-bound vesicles containing storage material that (a) (c) (b) Fig. 12.8.3 Macroscopic and microscopic appearances of the brain of a patient with Fabry disease. The patient died 15 years after a successful renal transplant, aged 62 years and with a cardiac pacemaker, of a dementing illness having suffered multiple stroke-like events. (a) CT examination of the brain demonstrating extensive white matter lesions. Cerebral atrophy characterizes the advanced stage of the neurological aspects of Fabry disease; ectopic calcification within the basal ganglia, cerebral cortex, and cerebellum is thought to locate to the media of small penetrating arteries. (b) Postmortem examination demonstrating cortical atrophy, ventricular dilatation, and white matter focal cavitation. (c) Histological examination of the brain demonstrating striking calcification of hypertrophic media of penetrating arteries, with associated leukoencephalopathy. Courtesy of Dr G. Alistair Lammy, Cardiff University.

12.8 Lysosomal disease 2135 may show a crystalline or concentric appearance, or—in the case of glycogen in Pompe's disease—vacuoles with a granular appearance. The appearance of concentric arrays of material strongly suggests a sphingolipidosis. Complete absence of platelet dense granules by electron microscopy is characteristic in Hermansky–Pudlak syndromes. Blood film examination Blood film examination is an often neglected but simple diag- nostic screening procedure that may indicate the diagnosis of a lysosomal disease. Amorphous material accumulates within the lysosomal vacuoles in the ceroid neuronal lipofuscinoses, mucopolysaccharidoses, and glycoproteinoses. In Chédiak–Higashi disease, giant granules are readily visible in neutrophils, eosinophils, and granulocytes. Smaller pathological bodies may be evident in circulating white blood cells and are typical of several mucopolysaccharide diseases, particularly MPS I (Hurler–Scheie), MPS VI (Maroteaux–Lamy), and MPS VII (Sly's syndrome) diseases in which granular deep lilac-staining inclusions (the Alder–Reilly abnormality) are readily detected in all leucocyte subtypes after staining by the Leishman method. Pathological vacuolation is prominent in peripheral blood lymphocytes in ceroid neuronal lipofuscinosis type 3 and Pompe's disease (the latter in which the vacuoles stain with periodic acid–Schiff reagent before, but not after, exposure to diastase). Lymphocyte vacuolation is reported in other lysosomal diseases (GM1 gangliosidosis, galactosialidosis, Salla disease, neuramin- idase deficiency, alpha mannosidosis, fucosidosis, I cell disease, and Niemann–Pick disease type A). Diagnostic biochemistry For most lysosomal storage diseases, the suspected diagnosis can be confirmed by biochemical studies. Storage compounds can, as in the case of the glycoproteinoses and mucopolysaccharidoses, be detected in the urine. Initial colorimetric screening methods may confirm the presence of elevated concentrations of glycosaminogly- cans but are not specific; chromatographic separation of individual glycoconjugates will assist further. More often, the diagnosis is established by confirming reduced or absent activity of the cognate lysosomal acid hydrolase. Specialized laboratories carry out panels of these assays depending on the clinical details provided by the clinician. Most assay

systems are based on the cleavage of synthetic fluorescent analogues of the natural substrate in question. Accurate clinical information greatly assists the laboratory in deciding which enzyme activity, of many, to assay. The usual sample is a peripheral blood leucocyte preparation made from whole blood, although fibroblast cultures obtained from skin biopsies or biopsy specimens of other tissues may be required. For some conditions, including Gaucher's disease, Fabry's disease Pompe's disease and lysosomal acid lipase deficiency, accredited la- boratories offer diagnostic assays based on dried blood spots on card. These developments, partly initiated and funded by the pharma- ceutical industry, have the advantage of convenience for transport to the diagnostic lab in a stable form, but the diagnostic performance and reliability compared with conventional fluid-phase assays car- ried out on fibroblast suspensions or blood leucocytes has yet to be completely established. There is increasing interest in development of newborn screening for lysosomal diseases, but so far this has been limited to a few specific geographic regions, such as Krabbe's disease in Illinois, Kentucky, Missouri, New York, Ohio, Pennsylvania, and Tennessee (all United States of America) and Taiwan (particularly for Pompe's disease). The biochemistry laboratory has a further role in determining the presence and concentration of markers of disease activity. Such biomarkers play an increasing role in clinical management, pharmaceutical development, and research. Markers in clinical practice include chitinase, chitotriosidase, and the chemokine CCL18/PARC as markers of the presence and extent of tissue in- filtration by the eponymous cell in Gaucher's disease. Particular lipid substrates (including lysolipids), in addition to their puta- tive mechanistic role, are used as potential biomarkers for diag- nosis and monitoring therapeutic effects: the unacylated, partly water-soluble congeners of the primary storage glycosphingolipid, lysoglobotriasolceramide, and ß-glucosylsphingosine are in- creasingly used in the clinical monitoring of Fabry's disease and Gaucher's disease, respectively. Molecular diagnosis Molecular analysis of genes encoding lysosomal enzymes may often support the enzymatic diagnosis, and may, on occasion, provide a rough prediction about the behaviour of the disease. DNA-based studies are of particular value for future prenatal diagnosis in a par- ticular pedigree, and for the diagnosis of carrier status in at-risk fe- males for heterozygosity in the X-linked diseases such as Hunter's, Danon's, and Fabry's diseases. In the last 20 years, there has been a strong and justified trend in favour of specific enzymatic and genetic diagnoses, rather than those based on the examination of biopsy material by light micros- copy with or without the additional use of special histochemical stains. Ultrastructural examination of biopsy material may be of particular value in recognizing the type of disorder but is rarely cru- cial for a specific diagnosis. Hitherto, histochemical and histopatho- logical methods have led to diagnostic inaccuracies, but it must be admitted that many cases of lysosomal disease—particularly as they affect adults—have in the past come to light as a result of bone marrow examinations, liver and muscle biopsies, and other Fig. 12.8.4 Sural nerve biopsy stained with toluidine blue from a patient with metachromatic leukodystrophy. Note the brown-staining granular material within Schwann cells and perineurial macrophages typical of this disorder due to the deposition of the glycolipid sulphatide. Courtesy of Dr J. Xuereb, Addenbrooke's Hospital.

 procedures carried out in an attempt to arrive at a diagnosis in an otherwise puzzling condition. High-throughput diagnostic DNA sequencing is now in use in several laboratories, either in the form of single gene sequencing to address a unitary suspected diagnosis or 'panels' of genes associated with a clinical presentation of interest. Research initiatives such as the '100,000 Genome Project' in the United Kingdom aim to enu- merate sequence variants and predict the causative mutations in rare or unexplained metabolic conditions.

Whole-exome sequencing is also gaining traction in the practice of metabolic diseases, especially among paediatricians, but attribution of causation to innumerable sequence variants that emerge from such broad searches remains a considerable logistical challenge and many ‘diagnoses’ cannot be validated. Treatment Supportive and palliative therapy No specific or curative treatment is available for most of the lyso- somal disorders and as a consequence, the psychological and social burdens are pervasive. As discussed earlier, the organ response to the metabolic defect is often stereotypical and similar to that seen in other diseases, with treatment limited to those supportive and pal- liative measures shared with other chronic diseases. Occasionally, organ transplantation is required to deal with heart, liver, or kidney failure. Orthopaedic surgical techniques, such as joint replacement surgery and stabilization of kyphosis using Harrington rods, are frequently required and beneficial. Patients with obstructive hydro- cephalus benefit from the placement of shunts for cerebrospinal fluid. Middle ear effusions and glue ear are also treated convention- ally with grommets. Physiotherapy for restricted joint movement and muscle weakness is valuable. Mobility aids and ventilatory sup- port add to the range of expensive and invasive measures required in the absence of definitive treatment. The search for specific treatments Lysosomal diseases have been the focus of several prominent therapeutic discoveries. The cooperation of informed patient groups, applied medical research funded by government organiza- tions, and the commercial interest of medium-sized pharmaceut- ical companies has been promoted by the introduction of Orphan Drug legislation. First enacted in the United States of America in 1983, and adopted in principle in Europe in 2001, the legisla- tion has facilitated the early exclusive licensing of products for rare diseases and has greatly enhanced corporate pharmaceutical investment. Orphan diseases are variously defined as those affecting fewer than 1 in 2000 of the population (Europe) or fewer than 200 000 individuals (United States of America); each lysosomal disease is, in effect, an ultra-orphan disorder, that is, a disease affecting fewer than 1 in 50 000 individuals. Despite attracting great attention as a result of the high individual costs of treatment, the total national burden of treatments for these diseases in countries with devel- oped healthcare systems is low (in England, the costs of specific treatments for lysosomal diseases amounts to about 0.1% of the health budget). Orphan drug development in lysosomal diseases: fortunes and misfortunes Encouraged by the success of several ‘blockbuster’ products such as recombinant human growth factors and erythropoietin, the orphan drug industry has grown rapidly. An early adopter of these oppor- tunities, Genzyme Therapeutics, the corporation that first jointly de- veloped macrophage-targeted enzyme therapies with the National Institutes of Health in the United States of America, and with sub- stantial financial support from the National Gaucher Foundation, introduced highly effective treatment for this disease within the aegis of the Orphan Drug Act. By 2009, alglucerase (Ceredase) followed by imiglucerase (Cerezyme) for Gaucher’s disease, supplied 5000 patients in more than 90 countries. The company, then the third lar- gest biotechnology corporation, reported revenues of about $4 bil- lion, of which about one-third were due to Cerezyme; revenue from agalsidase alfa (Fabrazyme) for Fabry’s disease was $424 million. Commercial success on such a scale supported continuing invest- ment in even more challenging disorders, including Pompe’s dis- ease. Delivery of the therapeutic protein Myozyme (recombinant human acid α-glucosidase (maltase)) to a large bulk of diseased skel- etal muscle, to which it is targeted by surface expression of mannose 6-phosphate residues, was a major challenge. The therapeutic de- livery required administration of gram quantities of the remodelled recombinant glycoprotein at each infusion. The scale of the manu- facturing resources required for the developmental Pompe’s disease clinical trial programme led to a lack of secure reserve stocks of the corporation’s leading products. By mid 2009, a vesiviral infection impaired the viability of the re- combinant Chinese

hamster cells in the bioreactors that synthesize Cerezyme and Fabrazyme. Manufacture was rapidly shut down with the immediate consequence of a ‘global supply restraint’ in which treatment for Gaucher’s disease and Fabry’s disease became critically limited. Many patients were without treatment for nearly 2 years. Fortunately, licensed products already in development were accelerated through the industrial scale-up and regulatory approval processes, and with expanded compassionate use and ac- cess programmes the manufacturing void was gradually filled. Of note, the Genzyme corporation was purchased by Sanofi and full- scale manufacturing and its global supply capacity was restored by late 2012. This biopharmaceutical shut-off of therapeutic supply was un- precedented in scale and totally unexpected. The episode revealed an inevitable but concealed risk associated with orphan drug legis- lation. While the orphan drug initiative serves as a powerful and successful incentive for drug development in neglected diseases with clear unmet needs, the rewards for market authorization of a first-in-class orphan agent with demonstrable efficacy and justifiable safety are essentially anticompetitive. Beyond marketing credibility for any given company and the bio- pharmaceutical industry overall, there are deeper consequences of the events described: (1) the episode should instruct all stakeholders of the need for any manufacturer to support and maintain adequate reserve stock, with shared costs as a result; (2) the value of sustained competition in therapeutic development, even in the realm of ultra- rare; and (3) the intellectual domination of the global community as a consequence of ‘life-changing’ enzyme therapy in Gaucher’s dis- ease opened up and exposed a vulnerable global patient community

 to the risk of corporate collapse and treatment withdrawal, although this nightmare scenario was avoided in this instance. Current therapeutic landscape At present, about 20 recombinant human enzyme preparations are in use or in late clinical investigation. Several companies are ex- panding interest in this rarefied field, with additional recombinant proteins (including biosimilar, modified, and semisynthetic mol- ecules), small-molecule products, and even gene therapy in robust competitive development (Table 12.8.2). The magnification of interest that has accompanied successful medical research into this area has generally been a model of utility and progress. It continues to provide for many patients and their families the hope that definitive relief might be forthcoming. Nevertheless, given the high cost per patient, decision-making bodies such as the United Kingdom National Institute for Health and Care Excellence (NICE) continue to look closely at the health eco- nomic benefits of individual treatments for rare diseases, operating as they do in healthcare systems that are financially constrained. Specific treatments and their mechanisms Augmentation of deficient activity Early experiments by Elizabeth Neufeld and colleagues using fibroblasts in which glycosaminoglycans accumulate due to mucopolysaccharidoses such as Hurler’s disease (MPS I, autosomal re- cessive) and Hunter’s syndrome (MPS II, X-linked), showed that the rate of degradation—rather than the rates of synthesis or secretion—of 35S sulphate-labelled substrate is severely disrupted. When (initially as a result of a laboratory error) fibroblasts obtained from these genetically distinct storage disorders were co-cultured, the pathological accumu- lation of glycosaminoglycans in lysosomes was prevented. The biosyn- thetic labelling technique was also used to show that degradation of the substrates was restored to normal in these co-culture experiments. Further investigation of this phenomenon by the Neufeld group demonstrated that each of the fibroblast cultures elaborated and de- livered a specific corrective factor to the medium, which ultimately proved to be a high molecular weight form of the hydrolases that were specifically lacking in fibroblasts from the corresponding disease. These corrective factors were identified in several comparable experi- ments using fibroblasts derived from other mucopolysaccharidoses and also several different

classes of lysosomal disease; when taken up from the medium, the factors restore the impaired intracellular degradation of cognate substrates. Functional correction of the biochemical defects thus permitted an early classification of distinct complementation groups among the MPS syndromes, often before the individual enzymatic defects had been characterized. Specific receptor pathways for the biosynthesis and uptake of nascent lysosomal proteins during the course of organelle biogen- esis have been identified: the process is usually brought about by the so-called recognition marker, mannose 6-phosphate. This ter- minal sugar is generated by a specific mechanism involving two post-translational modifying enzymes during the biosynthesis of soluble glycoproteins destined for the lysosomal matrix. Receptors, serving as intracellular lectins for mannose 6-phosphate ligands are densely expressed on prelysosomal membranes and mediate uptake of suitably labelled nascent proteins into the developing or- ganelle. However, this trafficking process is not foolproof and 10 to 20% of newly formed soluble lysosomal proteins are misdirected to the plasma membrane from which they are released; by the same token, an appreciable population of cation-independent mannose 6- phosphate receptors is expressed on the plasmalemma, serving the function of regulatory uptake of IGF. The ‘leakiness’ of this targeting system represents a default pathway for lysosomal protein secretion and recapture—as well as mutual complementation between dif- ferent cells and tissues. Functional complementation of lysosomal storage disorders by supplying particular molecular isoforms of the enzymes that are deficient in individual diseases provides a scientific justification for enzyme replacement treatment. Successful application of enzyme- replacement is dependent on an understanding of glycoprotein chemistry, receptor-mediated endocytosis, and the molecular cell biology of lysosomal biogenesis: identification of the secretion and recapture mechanism has provided further practical underpinning. The mannose 6-phosphate pathway is not the only mechanism for delivering proteins to the lysosome; indeed, the first successful en- zyme replacement therapy for Gaucher’s disease employed human glucocerebrosidase that was modified specifically to reveal ter- minal unphosphorylated mannose residues that greatly enhanced delivery of the therapeutic protein to cells of the macrophage lin- eage that are the principal focus of the disease. Characterization of lysosomal recognition markers occurred at a time when other cell surface glycoprotein recognition systems were being identified: the asialoglycoprotein receptor, the first mammalian lectin identi- fied by Ashwell and Morell, can mediate the uptake of modified plasma proteins by parenchymal liver cells in vivo. Recent studies show that in some cells, for example, in the inner ear and brain, as well as lymphocytes (but not fibroblasts or macrophages), delivery of nascent acid glucocerebrosidase to lysosomes is dependent on a unique tissue-specific chaperone function supplied by a lysosomal membrane protein (LIMP2). Mutations in the human LIMP2 gene appear to account for some atypical cases of Gaucher’s disease with neurological manifestations (including myoclonic epilepsy), kidney disease, and perplexing enzymology when examined in periph- eral blood cells and cultured skin fibroblasts; in these patients, acid glucocerebrosidase deficiency occurs in fibroblasts but not leuco- cytes or tissue macrophages. Haematopoietic stem cell transplantation Cellular complementation, by providing a source of wild-type enzyme delivered from allogeneic bone marrow transplantation, has also had spectacular successes in several lysosomal disorders. In Gaucher’s Table 12.8.2 Lysosomal diseases with approved enzyme therapies Disease with approved therapy Disease with trial completed but drug not approved Acid lipase deficiency Anderson–Fabry disease Gaucher disease types I and III α-Mannosidosis MPS type I MPS type II MPS type IVA MPS type VI MPS type VII Neuronal ceroid lipofuscinosis type II Pompe disease Krabbe disease (peripheral) Metachromatic leukodystrophy (intrathecal) MPS type IIIA (intrathecal)

section 12 Metabolic disorders 2138 disease, where the pathogenic cell is of haematopoietic origin, bone marrow transplantation was effective in the past. Successful engraft- ment led to full correction of the biochemical defect and reversal of most of the visceral and haematological effects of the condition that had not already progressed irrevocably. Now that a safer treatment in the form of enzyme replacement is available, bone marrow trans- plantation, with its attendant risks, is very rarely indicated. In diseases due to deficiency of soluble hydrolases, donor cells that repopulate the microglia (the brain equivalent of tissue macro- phages) may participate in the secretion-recapture mechanism, and in this form of cell replacement therapy would be expected to pro- vide a source of enzyme to vicinal cells. Haematopoietic stem cell transplantation has shown efficacy at an early stage of disease in several of the neurodegenerative lysosomal disorders, such as Hurler's disease (MPS I); very young infants, par- ticularly in the immediate neonatal period, with Krabbe's disease and, as described previously, in chronic neuronopathic Gaucher's dis- ease. As a result of improved outcomes of the intervention in general, treating physicians are re-evaluating the potential of haematopoietic stem cell transplantation in conditions where its role had previously been questioned, such as Hunter's syndrome (MPS II). Gene therapy Gene therapy has long been discussed in relation to lysosomal dis- eases since the capacity to transduce a focus of cells using vectors expressing the deficient enzyme or protein within a tissue is an at- tractive possibility for sustained functional complementation. This approach has shown spectacular benefit in several different spontan- eous and transgenic animal models that are genetically and clinically coherent with their human counterparts. At present, two principal stratagems—both dependent on viral vectors—are being explored in lysosomal diseases; mainly those with life-threatening features including neurological disease. Third-generation lentiviral vectors are able to transduce dividing cells such as haematopoietic stem cells and integrate into the host- cell nuclear genome. Adeno-associated viral vectors do not integrate into the nuclear DNA but remain episomal and direct biosynthesis of therapeutic transgene products in nonmitotic cells, including neural cells. In the case of haematopoietic stem cells, the genetically cor- rected autologous cells of haematopoietic origin can be reinfused into the donor after transduction ex vivo. Here after engraftment they are able to deliver the corrective protein function (wild-type enzyme) to the tissues in circumstances where these migratory cells of haemato- poietic origin slowly populate the sites of disease, such as the brain and spinal cord. It is believed that they give rise to macrophages and lymphoid cells with the potential to secrete corrective factors for up- take and functional complementation of local disease. Reconstitution of bone marrow-derived cells, engineered by lentiviral gene transfer to overexpress the wild-type enzyme (in- tended to deliver an abundance of soluble enzyme), are being ex- plored and efficacy has been shown in late-infantile and juvenile metachromatic leukodystrophy. Retroviral vectors and gene con- structs were used to introduce the desired DNA sequence encoding arylsulphatase A into autologous explanted haematopoietic stem cells of young, presymptomatic subjects with metachromatic leukodystrophy, and the genetically corrected cells were expanded in culture and then returned to the patient's circulation. Phase I/II clinical trial results reported by Dr Alessandra Biffi and colleagues in Milan provide convincing evidence of some neurological benefit or 'rescue' compared with historical and sibling control patients with early-onset disease not so treated. It is not certain whether transplantation of haematopoietic stem cells from healthy matched related donors give comparable or inferior results. Despite the dif- ficulty in determining efficacy directly in the gene therapy studies, remarkably the disease did not manifest or progress in the first eight of nine patients who underwent the autologous gene therapy pro- cedure. What is clear, however, from worldwide experience not only of haematopoietic stem cell transplantation but genetically modified autologous stem cell therapy, is that compelling

clinically signifi- cant benefit is almost solely restricted to recipients who undergo the intervention in the presymptomatic phase of this disease. The other stratagem under active development for clinical applica- tion in the lysosomal diseases employs vectors based on the use of re- combinant nonpathogenic ‘passenger’ adeno-associated picornavirus of several serotypes with preferential ability to transduce certain cell types, known as ‘tropisms’. These tropisms can be harnessed to facilitate delivery and expression of the cognate therapeutic transgene DNA to particular tissues such as the liver or neural cells. Each vector system has potential advantages and shortcomings, which are discussed in the suggested ‘Further reading’ material at the end of this chapter. At the time of writing, several trials using direct injection of re- combinant adeno-associated viral vectors expressing the cognate corrective human proteins into the brains of children with two other neurological lysosomal diseases have been safely completed. Early safety and efficacy outcomes of the Sanfilippo’s disease type A (MPS IIIA) trial using rAAV rh10 have been reported: the safety criterion was met with an indication of stabilization in three of the four patients and possible improvement in one. Following the recent licensing of the vector, a more definitive phase III trial is planned. Encouraging outcomes of a phase I/II trial using intracranial rAAV vectors serotype 5 in four children aged 20 to 53 months with Sanfilippo’s disease type B (MPS IIIB) have been reported. Not only were the safety requirements met, but over 24 months neurocognitive progression was improved in all patients compared to that expected. N-acetylglucosamine activity was detected in lumbar cerebrospinal fluid and was 15 to 20% of that in unaffected children. Other phase I/II clinical trials are in active de- velopment for other lysosomal diseases. Enzyme replacement therapy Discovery of the mechanism by which lysosomal proteins are delivered to the nascent organelle have gone hand-in-hand with the hope of treat- ment based on the targeting of therapeutic enzymes to diseased tissues. The first commercial preparation of glucocerebrosidase (alglucerase, Ceredase) was not licensed until 1991 and 1994 by the Food and Drug Administration (FDA) in the United States of America and by the European Medicines Agency, respectively, after decades of painstaking research. This was purified from placentae and its glycan structure modified enzymatically to reveal terminal mannose groups that bind the mannose receptor on cells of macrophage origin. It mitigated many features of Gaucher’s disease when given parenterally. The therapeutic and commercial success of alglucerase, along with potential difficulties in maintaining the supply of suitable placentae, stimulated the demand for a recombinant preparation (imiglucerase, Cerezyme). Expansion of the approach to include diseases that would be targeted using mannose 6-phosphate, the more familiar lysosomal recognition marker, followed.

12.8 Lysosomal disease 2139 Since 2001, recombinant protein therapies have become avail- able for Fabry’s disease (two products), Hurler–Scheie disease (MPS I), Hunter’s disease (MPS II), Maroteaux–Lamy disease (MPS VI), Pompe’s disease (glycogen storage disease type II) and Morquio’s dis- ease type A (MPS IVA), and more recently lysosomal acid lipase de- ficiency (Wolman’s disease, the infantile form, and cholesteryl ester storage disease, the later-onset form). Velmanase alfa (Lamzede) has recieved marketing authorisation by the European Medicines Agengy in 2018 for the treatment of Alpha-Mannosidosis. Trials of intra- thecal enzyme therapy are in progress for several lysosomal disorders that cause neurodegeneration, including MPS II and metachromatic leukodystrophy. Universal availability of these treatments is limited by their very high cost (licensed doses cost upwards of $200 000 per annum for an average adult) and by the requirement for a sophis- ticated healthcare infrastructure to support the delivery and moni- toring of the therapy. Thus, even imiglucerase (Cerezyme), which has been available for over 15 years and whose efficacy is clear, is available to fewer than 20% of patients globally for whom it is

indicated. Analogous biological products have now been approved for treat- ment of Gaucher's disease. Velaglucerase alfa (VPRIV) is produced by specific upregulation of the endogenous gene sequence in a fibro- sarcoma cell line cultured in the presence of an inhibitor of post- translational glycosylation to express human glucocerebrosidase decorated by high-mannose glycans. After an extensive clinical trial programme and aided by the supply limitation of imiglucerase, it received marketing approval in 2010. Taliglucerase alfa is produced using recombinant technology in cultured carrot cells and has re- ceived authorization as Elelyso by the FDA in the United States of America (but not by the European Union). Two enzyme products are approved by the European Medicines Agency in Fabry's disease. Agalsidase alfa (Replagal) is generated by targeted overexpression of the human AGAL gene in a human fibro- sarcoma cell line and is marketed at a dose of 0.2 mg/kg every other week. Agalsidase beta (Fabrazyme) is a traditional recombinant product generated in Chinese hamster ovary cells and marketed at a dose of 1 mg/kg every other week. Agalsidase alfa did not secure marketing approval in the United States of America. Development continues of an alglucosidase modified by the chemical addition of mannose phosphate ligands: avalglucosidase alfa is in phase III trials in Pompe disease. A clinical trial programme in Fabry's disease using an agalsidase modified by addition of poly- ethylene glycol adducts—pegylation—has been initiated, with evi- dence of different biodistribution and cell uptake characteristics. The therapeutic position of newer enzyme products for other con- ditions will evolve, but it must be recognized that in general the con- ditions for which they are licensed are heterogeneous and, it appears, more intractable than the visceral and haematopoietic features of Gaucher's disease. It seems likely that internalization of glycoproteins decorated by mannose 6-phosphate signals is less rapid and effective in vivo than the uptake and delivery of those displaying terminal man- nose residues recognized by the mannose receptor on macrophages. Pharmacological chaperone therapy This stratagem is based on the ability of small molecules to bind to key regions of mutant proteins that are misfolded and thus prema- turely degraded in the endoplasmic reticulum and Golgi network. Aberrant protein folding is increasingly recognized as a molecular mechanism in inherited diseases since, as a result of disrupted cotranslational processing, it leads to an operational deficiency of protein function at the point of action. Pharmacological chaperones are molecules which bind to mutant proteins in a stable complex and thus assist delivery to the site of action in the correct cellular compartment. In the case of lysosomal enzymes, the candidate chaperone molecule is usually a competitive inhibitor of the nascent enzyme at neutral pH and designed to dis- sociate from the mature lysosomal enzyme on arrival at the acidic environment of the organelle. Pyrimethamine, a licensed oral antiprotozoal folate antagonist, has chaperone-like effects in cells harbouring some HEXA muta- tions from some patients with attenuated forms of Tay–Sachs and Sandhoff's diseases. This drug, which traverses the blood–brain barrier, has undergone small phase I/II clinical trials in which its efficacy has mainly been disappointing. However, the authors have seen one patient with juvenile Sandhoff's disease in whom pyri- methamine induced a large increase in enzyme activity in cultured skin fibroblasts, with more mature hexosaminidase protein in the lysosomal compartment together with partial clearance of the cog- nate substrate (GM2 ganglioside); improved cognitive power and neuropsychological test scores were seen on oral exposure to the drug (with calcium folinate supplements) over a 5-year period. Several iminosugars correct the misfolding of mutant lysosomal glucocerebrosidases in experimental cell systems, including cul- tured fibroblasts obtained from Gaucher's patients: one of these, isofagomine, underwent clinical evaluation but failed to demonstrate sufficient efficacy. Equally, clinical trials of single-agent chaperone- based therapy in Pompe's disease failed to show benefit. Clinical trials have completed with another iminosugar (1-

deoxygalactonojirimycin, migalastat) in patients with Fabry's disease in whom in vitro studies indicate the potential for functional enhancement of several mutant α-galactosidase variants. One trial in treatment-naïve subjects dem- onstrated a modest reduction in storage vacuoles on kidney biopsy. In a separate switch study in patients already receiving enzyme therapy, subjects' kidney function remained stable. In both studies, interesting and potentially salutary effects were noted on left ventricular mass by echocardiography. On the basis of these findings, migalastat received marketing authorization in the European Union in 2016 and in the United Kingdom has also received approval by NICE for reimburse- ment. Phase three trials are in progress to examine a combination of a chaperone and a second-generation enzyme therapy for Pompe dis- ease. In this case the aim of the chaperone is to act extracellularly to improved the stability and delivery of the therapeutic enzyme. Although the use of pharmacological chaperones is an attractive concept for the oral treatment of lysosomal diseases, hitherto— outside the use of neopterin in phenylketonuria and other vitamin cofactors such as pyridoxine for homocystinuria—no small mol- ecule has shown true clinical efficacy in a putative misfolding disease. Restriction (or rebalancing) of substrate flux ('substrate reduction therapy') For many years, the accumulation of storage material within lyso- somes has been considered to be the precipitating factor for the development of tissue injury and the inflammatory response that accompanies the lysosomal storage disorders. By analogy with the development of atherosclerosis due to impaired catabolism of chol- esterol bound to low-density lipoproteins, it is principally a failure

 of degradation or export from the lysosome that leads to the patho- logical storage. Thus, like the statins which inhibit the first committed step in the biosynthesis of cholesterol, the concept of depleting the supply of macromolecular substrate to prevent the accumulation of injurious material has been developed experimentally and brought to clinical practice in the sphingolipid disorders. Two classes of inhibitor are prominent in therapeutic studies: iminosugars derived from naturally occurring compounds (acting principally as sugar mimetics) and synthetic pyrrolidino com- pounds (acting as analogues of the ceramide moiety of sphingolipids). Iminosugars Some iminosugars related to deoxynojirimycin are inhibitors of the ceramide-specific UDP-glucosyltransferase reaction as the first committed step in the biosynthesis of glycosphingolipids. Following experimental studies in cultured cells with pathological storage of glycolipids in lysosomes and in murine models of debilitating human glycosphingolipidoses, clinical trials of N-butyldeoxynojirimycin (miglustat, Zavesca, a particular analogue of iminosugars) were conducted in in Gaucher's disease. Evidence of disease regression was obtained in an open-labelled trial with reduction in visceral en- largement, enzymatic markers of Gaucher's disease activity (plasma chitotriosidase activity), and a slow improvement in haematological parameters. The drug gained marketing authorization in 2002 as an orally active second-line treatment for type 1 Gaucher's disease in adults unable or unwilling to receive enzyme therapy. Although ap- proved as a first-in-class inhibitor of substrate biosynthesis, further research now suggests that among the off-target effects of miglustat, its action as a more potent inhibitor of a neutral glucocerebrosidase involved in sphingolipid recycling contributes to its therapeutic ef- fects in non-neuronopathic Gaucher's disease. Since the iminosugars are small molecules with the potential to penetrate the blood–brain barrier, the possibility of their use (either as a monotherapy or as a synergistic treatment with enzyme therapy) for neuronopathic Gaucher's disease has been raised, as well as for the treatment of the otherwise intractable glycosphingolipidoses that cause severe neurological disease. However, miglustat was found not to be effective in young patients suffering from the neurological ef- fects of type 3 (chronic neuronopathic) Gaucher's disease who were receiving

imiglucerase, but it appeared to improve pulmonary mani- festations which usually fail to respond to enzyme therapy alone. Miglustat has also received marketing authorization in Europe for treatment of Niemann–Pick disease type C, having shown delay in progression of the neurological features in a randomized, open-label clinical trial. Synthetic pyrrolidino compounds Eliglustat, a pyrrolidino compound that is a ceramide analogue with a highly selective and potent inhibitory action on glucosylceramide biosynthesis, has demonstrated strong therapeutic effects in non-neuronopathic Gaucher's disease. Encouraging 4-year phase III and 8-year phase II clinical studies and 1400 patient-years of clinical trial exposure support its position for most adult patients with type 1 (non-neuronopathic) Gaucher's disease as an alternative first-line agent to enzyme therapy. The oral activity in once- or twice-daily dosing, represents an important advantage over intravenous infu- sions of enzyme therapy given every 2 weeks. Prescription requires specialist monitoring, with dosing guided principally by cytochrome P450 (CYP) genotyping since the agent is extensively metabolized by the CYP2D6 and to some extent CYP3A4 systems. Eliglustat (as Celderga) has received marketing authorization by the FDA in the United States of America as an oral first-line agent for adults with type 1 (non-neuronopathic) Gaucher's disease, is authorized by the European Medicines Agency, and is approved by NICE for reim- bursement by the National Health Service (as in many other regions). Eliglustat is a substrate for the P-glycoprotein MDR1 efflux transport system, and thus does not distribute effectively to the brain. With the recognition that there remains a large need for small molecule drugs that traverse the blood–brain barrier to exert therapeutic effects in those lysosomal diseases with neurological effects, and that the biosynthesis of glucosylceramide represents a common target for several disabling sphingolipid disorders, the Sanofi Genzyme company has identified an additional inhibitory molecule, venglustat, for clinical conditions requiring systemic and/or neurological targeting. A phase I/II clinical trial of this agent has been completed in Fabry's disease, and similar trials are underway in adults with type 3 (chronic neuronopathic) Gaucher's disease and in patients with Parkinson's disease who are heterozy- gous for pathological mutation in the GBA1 gene. Given the bio-chemical relationships of glucosylceramide with the gangliosides that accumulate in Tay–Sachs and Sandhoff's diseases, as well as GM1 gangliosidosis, chronic forms of these neurological diseases are potential therapeutic targets for this drug class. Examples of lysosomal disorders
Gaucher's disease This disorder may occur at any age and has been regarded as the most frequent of the lysosomal storage diseases, although recent evidence suggests that Fabry's disease, particularly in its attenu- ated forms, is substantially more frequent. The condition is usu- ally due to a catalytic deficiency of glucocerebrosidase, although rare cases of deficiency of its cognate sphingolipid activator protein (SAP-C) may cause a severe disorder usually similar to the subacute neuronopathic form of true Gaucher's disease. Numerous mutations responsible for the enzymatic deficiency have been identified in the human glucocerebrosidase gene and the reader is referred to the specialist literature for those genotype/phenotype correlations that broadly apply to this protean disorder. The mature protein contains 497 amino-acid residues; a 39 amino-acid lead peptide is cleaved from the initially-translated polypeptide that contains 536 initial amino acids. A recent change in nomenclature now considers the position of amino-acids relative to the beginning of the 536 amino- acid peptide, rather than the 497 amino-acid mature protein. Thus the most frequent mutations, known previously as N370S and L444P, are now referred to as p.Asn409Ser and p.Leu483Pro respectively. Gaucher's disease type 2 and type 3 Rarely, infants are born with an almost complete lack of gluco cerebrosidase activity: they die within a few days of birth or are still-born due to skeletal deformities and/or dehydration as a result of loss of skin integrity (collodion babies). Infantile Gaucher's disease (classified as acute neuronopathic or type 2 disease) is a rare

neuronopathic disease with bulbar palsy, opisthotonus, and minor visceral enlargement. It is invariably fatal

12.8 Lysosomal disease 2141 in the first 2 years of life and does not respond to either systemic or intrathecal enzyme replacement therapy. While neurological disease may occur in children, adolescents, and young adults with Gaucher's disease, it is less severe than in the infantile variant and is termed subacute neuronopathic or type 3 dis- ease. In such patients the disease is associated with supranuclear gaze palsies, ataxia, nerve deafness, myoclonus, and (occasionally) seizures. In type 3 disease the neurological condition usually deterior- ates slowly but is exacerbated if splenectomy is performed for the accompanying splenomegaly and associated pancytopenia. Where possible, and with vigorous enzyme therapy, splenectomy is best avoided, although partial splenectomy may be carried out to ameli- orate pressure effects and life-threatening thrombocytopenia. Subacute neuronopathic disease is not always fatal and often improves with bone marrow transplantation and enzyme replace- ment therapy, although the effects of the latter are restricted to the systemic, non-neurological aspects. Affected children may show striking visceromegaly, with the associated gaze palsies often playing a small part in the clinical presentation. Although juvenile subacute neuronopathic Gaucher's disease (type 3) occurs sporadically in all populations, there is a small iso- late in Northern Sweden where all individuals are homozygous for a single point mutation in the glucocerebrosidase gene (L444P) that has arisen by descent from a common ancestor. Gaucher's disease type 1 The most frequent form of Gaucher's disease is the so-called adult non-neuronopathic form (type 1). This is found in all populations but is over-represented in Jews of Ashkenazi origin. Although the condition does not commonly affect the nervous system, visceral and skeletal manifestations are prominent.
Clinical features Characteristically, Gaucher's disease presents with pancytopenia, with bleeding due to thrombocytopenia and splenic enlargement. Acutely painful episodes also occur in the bones, particularly during growth, and these episodes are followed by the evolving MRI appearances of osteonecrosis with consequential effects on the integrity of large joints, including the hip, knee, and shoulder (Fig. 12.8.5). The increased frequency of infarction events is an important aspect of Gaucher's disease that, as yet, has not been ex- plained, and bone necrosis remains an aspect of the condition that often persists despite enzyme therapy. In the era before enzyme replacement therapy, splenectomy was often carried out during childhood to relieve the pressure effects of the en- larged organ and to ameliorate the effects of accompanying cytopenias. Although there appears to be a striking temporal association between splenectomy and the development of severe bone disease, it is unclear as to whether this is directly due to the effects of the splenectomy or the consequential manifestations of disease severity. Nonetheless, splen-ectomy is best avoided where at all possible. Splenectomy in Gaucher's disease carries a greatly enhanced risk of overwhelming infection; this includes infection with protozoa, such as babesia and malaria, as well as capsulated bacteria, for example, Streptococcus pneumoniae, Haemophilus influenzae, and Neisseria meningitidis. In addition to the effects of osteonecrosis, the osseous manifest- ations of Gaucher's disease are very diverse and include the presence of expanded bone lesions (Fig. 12.8.6) with surrounding cortical Fig. 12.8.5 T2-weighted MRIs obtained from the lower femur and upper tibia of a 30-year-old woman with non-neuronopathic Gaucher's disease experiencing pain due to acute avascular necrosis of bone. Note the geographical areas of increased signal intensity on the T2-weighted image due to increased tissue water representing oedema surrounding the necrotic tissue. Courtesy of Professor D. Lomas, Addenbrooke's Hospital.
Fig. 12.8.6 Expanded lytic lesion at the distal end of the femur in a 44-year-old woman with severe

Gaucher’s disease complicated by osteoporosis, osteonecrosis, and, as shown, expanded lytic lesions in long bones leading to local infiltration of the marrow space by Gaucher tissue.

section 12 Metabolic disorders 2142 thinning related to Gaucher cell infiltrates within the bone marrow (‘Gauchomas’). Diffuse osteoporosis accompanied by pathological fractures may also compound the skeletal manifestations. Kyphosis due to crush fractures of vertebrae are common in untreated adults, particularly in postmenopausal women. Gaucher’s disease may rarely be associated with pulmonary infil- trates, including reticulonodular opacities, restrictive lung defects, and various abnormalities of the pulmonary circulation, causing pulmonary hypertension. The hepatopulmonary syndrome, ac- companied by platypnoea and associated with severe scarring liver disease or cirrhosis and portal venous hypertension, has also been reported in severely affected adults. In its untreated state, Gaucher’s disease is a miserable condi- tion leading to progressive skeletal deformity, pancytopenia, and visceral enlargement with failing organ function punctuated by painful visceral bone crises. The mean age of death in a single large series reported from Pittsburgh, Pennsylvania, was 60 years during the pretreatment era, but this does not take into account the poor quality of life of most affected individuals. Some homo- zygotes for ‘mild’ missense mutations in the glucocerebrosidase gene (especially the widespread mutation, N370S) may escape detection and remain asymptomatic throughout a long adult life. Detailed investigation reveals only a mild thrombocytopenia and trivial splenomegaly in some cases. However, monoclonal gammopathy is frequently present after the age of 45 years. It is uncertain as to what extent the presence of such mutations in the population at large (homozygosity for N370S occurs in about one in 960 Ashkenazi Jews) contributes to the development of β-cell lymphoproliferative disorders, such as B-cell lymphoma and mye- loma, in this at-risk group. Other clinical aspects Parkinsonism and Gaucher’s disease—a complex relation- ship Approximately 5% of patients develop extrapyramidal disease resembling parkinsonism in middle life. The response to dopaminergic agents is often less clear than in classical idio- pathic Parkinson’s disease and the disorder may progress more rapidly. This complication may reflect the emerging strong but ill-understood relationship between mutant glucocerebrosidase al- leles and Parkinson’s disease and especially Lewy body-associated diseases and α-synucleinopathies in several populations: heterozy- gous mutations in the gene encoding glucocerebrosidase represent the commonest single genetic association with Parkinson’s disease in all populations studied. It appears that there is at most a small gene-dosage effect and Parkinson’s disease appears to be little more frequent in patients with Gaucher’s disease than in their heterozy- gous parents. An international study by Sidransky and colleagues involved the search for two frequent missense GBA1 mutations, L444P and N370S, in patients with Parkinson’s disease attending 16 centres. A total of 5691 patients with Parkinson’s disease (780 Ashkenazi Jews) and 4898 control subjects (387 Ashkenazi Jews) were geno- typed. Among Ashkenazi Jewish subjects, either mutation was found in 15% of patients but only 3% of control subjects; among non-Ashkenazi Jewish subjects, either mutation was found in 3% of patients and less than 1% of controls. GBA1 was fully sequenced in 1983 non-Ashkenazi Jewish patients, and mutations were iden- tified in 7%, showing that limited mutation screening can miss half the mutant alleles. The odds ratio for any glucocerebrosidase mu- tation in patients with Parkinson’s disease compared with controls was 5.43. When compared with patients with Parkinson’s disease who did not carry a GBA mutation, those with a mutation had an earlier presentation with the disease, were more likely to have affected relatives, and were more likely to have atypical clinical manifestations. Relationship to B-cell malignancy Gaucher’s disease abnor- malities include a polyclonal immunoglobulin response

that may progress to monoclonal gammopathy, amyloidosis, or even frank myeloma and B-cell lymphoma, with an estimated 20- to 40-fold in- creased risk compared with heathy subjects without Gaucher's dis- ease. These malignant complications are now an important cause of death in adult patients with type 1 Gaucher's disease. Their cause appears to be related to the clonal expansion of B cells that specif- ically secrete antibodies directed against the pathological complex lipids and that are driven, at least initially, by a subclass of follicular B helper T cells (TFH2) recognizing the glycosphingolipids presented by the CD1d molecule. Other plasma and metabolic abnormalities Low-density lipopro- tein and high-density lipoprotein cholesterol fractions are abnormal in the plasma. Basal metabolic rate is increased. Some lysosomal enzymes are elevated, including tartrate-resistant acid phos- phatase, hexosaminidase, and a human chitinase, chitotriosidase. Chitotriosidase may reflect the severity of the disease and has proved to be very useful for monitoring Gaucher's disease activity in response to treatment. The enzyme is elevated, sometimes several hundredfold above normal, in the untreated condition. Pathology The pathognomonic abnormality is the presence of large storage cells, which are activated macrophages (Gaucher cells), typically found in the splenic sinusoids. The Gaucher cells (Figs. 12.8.7 and 12.8.8) replace the Kupffer cells of the liver, alveolar macrophages of the lung, and in the bone marrow. Fig. 12.8.7 Light micrograph of a Leishman-stained bone marrow biopsy obtained from a 23-year-old man with type 1 Gaucher's disease. Note that the large, pale-blue staining Gaucher cells with striated cytoplasm replace the Kupffer cells of the liver, alveolar macrophages of the lung, and of the bone marrow.

12.8 Lysosomal disease 2143 Diagnosis The diagnosis of Gaucher's disease is based on white-cell acid β-glucosidase activity, which may be accompanied by the elevation of one or more related marker enzymes such as chitotriosidase or tartrate-resistant acid phosphatase in the serum. Spleen tissue, liver biopsy material, or bone marrow aspirates may show the character- istic oligonucleate storage cells demonstrating striated cytoplasm on Leishman staining (Fig. 12.8.7), but which appear as pink sheets in tissue sections stained with haematoxylin and eosin. Molecular ana- lysis of the glucocerebrosidase gene may identify widespread mutant glucocerebrosidase alleles and may assist in the diagnosis and inves- tigation of family members at risk for this recessive disorder. Treatment Until recently, the treatment for Gaucher's disease was palliative. Bone marrow transplantation has been undertaken in a few infants and children with rapidly progressive disease, including those with the subacute neuronopathic form type 3. When successful, this may correct most of the systemic manifestations of the condition and restore growth, and some observers believe that it may arrest fur- ther neurological deterioration. However, bone marrow transplant- ation is no longer in routine use because of the accompanying severe risk resulting from the procedures and constraints in the supply of donors, especially MHC-matched, first-degree relatives. Steady im- provement in the outcome of stem cell transplantation in general raises the possibility that this stratagem could be re-evaluated in patients with chronic neuronopathic Gaucher's disease in circum- stances where health services resources will not sustain the long- term costs of high-cost molecular therapies (whether enzyme therapy or other potential chronic therapies should, for example, venglustat prove to be safe and effective). Enzyme augmentation therapy Enzyme augmentation therapy was introduced during the early 1990s in the form of a natural product extracted from the human placenta, alglucerase (Ceredase). The recombinant glycoform, imiglucerase (Cerezyme), is supplied as a lyophilized powder which is reconstituted for intravenous infusion, given most commonly every 2 weeks. After a few weeks of enzyme administration, most patients show an improvement in the blood parameters of disease

activity and a reduction of the chronic inflammatory response that accompanies the condition. The platelet count rises and there is correction of the hypersplenic blood picture, with reduction in hepatosplenomegaly. There is improvement in asthenia and quality- of-life measures. Similar salutary effects are noted with the use of the more recently licensed enzyme therapies. Since most patients express the protein antigen endogenously, hypersensitivity and immune reactions are very rare. Apart from the inconvenience of periodic intravenous infusions, treatment is well tolerated and many patients in Europe and the United Kingdom choose to take their treatment as self-administered infusions at home. Controversy remains as to the appropriate dose of enzyme therapy, whether in the form of imiglucerase or the newer products velaglucerase alfa or taliglucerase, but most authorities agree that administration of the enzyme should be lifelong. There are several schools of thought as to whether enzyme therapy should be admin- istered at a high dose to start with, perhaps then reducing as evidence of disease regression becomes clear, or whether a more variable but lower dose be given and altered according to response. Disease activity is assessed by objective parameters, including visceral en- largement, and by determination of surrogate biomarkers such as chitotriosidase and blood counts. The application of simple defined therapeutic goals with close monitoring of individual patients has much to recommend it. Achievement of key goals and amelior- ation of disease-associated parameters is more rapid when high- dose enzyme therapy is administered. In patients with the subacute neuronopathic form of the condition (type 3), international guide- lines suggest that a dose of at least 60 units of enzyme/kg bodyweight per month is necessary to secure disease regression. This is very ex- pensive, costing as much as £200 000 per year for an adult. Substrate reduction therapies Miglustat When taken for several months, miglustat (Zavesca; N-butyldeoxynojirimycin) appears to reduce the content of gan- gliosides in circulating white cells and has salutary effects on key laboratory and clinical parameters of Gaucher's disease activity. It is licensed in the United States of America and Europe for use in mild to moderate type 1 Gaucher's disease, albeit with certain restric- tions. Short-duration unwanted effects (including diarrhoea due to inhibition of intestinal disaccharidases) are frequent, although they usually respond well to dietary adjustments. The occurrence of per- ipheral neuropathy after long-term administration may restrict the indications for its use. A trial to determine whether or not Zavesca is inferior to maintenance therapy with Cerezyme in patients with type 1 Gaucher's disease after stable control of their disease who then switch to the oral agent has shown that for some patients the disease remains stable, but many discontinued the medication either as a re- sult of adverse effects or re-emergent features of disease. Eliglustat In the ENGAGE trial, 40 patients were randomized to eliglustat or placebo for 9 months. Salutary changes in the key parameters of spleen and liver volume, haemoglobin, and platelet count were observed, to the degree expected of enzyme therapy. In the ENCORE study, 160 patients were randomized to receive Fig. 12.8.8 Electron micrograph showing the cytoplasm of a Gaucher cell in the spleen of a 56-year-old man removed because of life- threatening thrombocytopenia and pain due to a recent splenic infarct. Note the vesicular spaces filled with fibrillary glycolipid storage material.

 eliglustat (n = 106) or continued enzyme therapy (n = 54) after at least 3 years of enzyme therapy. The trial met its primary endpoint demonstrating noninferiority of eliglustat in composite haemato- logical and visceral parameters over 12 months, and 4-year follow- up data showed safe stabilization in most patients. Use is restricted to individuals who are not ultra-rapid metabolizers of the drug by the CYP2D6 enzyme. Complex drug–drug interactions require careful prescribing practice, but safety and tolerability have been

ac- ceptable in the clinical trial programme, with no sustained or major safety concerns hitherto. Other aspects of treatment Treatment for Gaucher's disease should include appropriate immun- ization and antimicrobial prophylaxis in the fortunately diminishing number of patients who have undergone splenectomy. Osteoporosis may be an indication for bisphosphonate drugs. Patients may require joint replacement surgery to ameliorate the effects of bone infarction crises and, in rare instances, liver transplantation for end-stage liver disease. All surgical procedures carry a risk of haemorrhage in the face of thrombocytopenia, platelet dysfunction, or blood coagula- tion factor abnormalities. It is thus critically important to engage expert assistance from a haematologist in planning surgical inter- ventions. Bone marrow transplantation probably does not have a role today, except in rare circumstances. Evidence of metabolic bone disease complicating the disorder should be always sought and osteoporosis should be treated promptly with enzyme replacement therapy, with the additional consideration of orally active or parenteral bisphosphonates. Where present, a defi- ciency of 25-hydroxyvitamin D should probably be treated with ap- propriate supplements. Some patients develop deficiency of vitamin B12 and this should be sought for and treated promptly. On account of the increased risk of infection due to intrinsic chemotactic and phagocytic defects as well as splenectomy, patients with Gaucher's disease undergoing surgery or with systemic infec- tion should be promptly treated, preferably with parenteral anti- microbial agents. Fabry's disease Fabry's disease is an X-linked disorder, unlike many of the lyso- somal diseases, apart from Danon's and Hunter's disease (MPS II). Deficiency of α-galactosidase A causes the accumulation of ceramide trihexoside (otherwise known as globotriaosylceramide) and related compounds including the deacylated equivalent lyso- GB3, which principally derives from the breakdown of lipids present in senescent red cells. A notable feature is the presence of clinical signs and symptoms in most heterozygous female carriers of the condition. Although these manifestations are usually less severe and of later onset than in affected hemizygous males, florid and life- shortening clinical disease has often been observed (and ignored) in affected women. Clinical features and prognosis The most characteristic symptoms of the 'classical' or severe form of the disease, usually indicative of absent or very low enzyme activity, are the onset in early childhood of lancinating pain with background burning sensations in the extremities that are made worse by exercise and exposure to extremes of temperature. These attacks can be very disabling and represent neuropathic pain, which is difficult to control. The acroparaesthesias are often attributed to Raynaud's phenomenon, which is indeed associated with Fabry's disease, but this relationship is unclear. Nonetheless, many of the symptoms of Fabry's disease can be explained by neuropathy affecting autonomic nervous tone. Patients with Fabry's disease have disturbing gastrointestinal symptoms, characterized by diarrhoea shortly after eating; attacks of abdominal pain associated with unexplained fever also occur. These abdominal symptoms may also be related to autonomic neuropathy. Most men with established 'classical' disease notice a striking ab- sence of peripheral sweating, and often suffer erectile dysfunction. They often have a characteristic facial appearance (Fig. 12.8.9). High-tone loss of hearing is also a common feature of Fabry's dis- ease, which appears to reflect selective injury to cochlear neurons. Affected male hemizygotes have small, raised, red vascular skin le- sions (angiokeratomas) particularly around the buttocks and genital region (Fig. 12.8.10). These lesions are often detected in limited areas of affected heterozygous females and reflect X-chromosome inactivation patterns in the skin. With increasing age, progressive tubular, interstitial, and glom- erular disease leads to proteinuria and renal failure. Many patients require renal support, including haemodialysis, peritoneal dialysis, or kidney transplantation. Cardiac hypertrophy, especially of the left ventricle, occurs with conduction defects leading to a shortened PR interval and a pro- longed QRS complex, later accompanied by

tachyarrhythmias and complete heart block. Left ventricular hypertrophy may be associ- ated with functional limitation due to diastolic dysfunction. The use of MRI has drawn attention to typical patterns of fibrosis in the mid- wall of the myocardium in particular regions of the left ventricle. This fibrosis may progress in the absence of cardiac hypertrophy, particularly in women. It is associated with risk of arrhythmia and reduced response to specific therapy. There is an increasing recognition of variant forms of Fabry's disease, which appear to be predominantly manifested by cardiomyopathy—without the 'classical' acroparaesthesia, anhidrosis, and angiokeratomas—in older patients with appreciable residual α-galactosidase activity. Disease of capillaries and medium-sized vessels in the brain is associated with unusual microvascular changes, particularly in the posterior cerebral circulation, and also causes stroke. Disease expression in many carrier females, who may rarely de- velop renal failure, is often accompanied by angiokeratomas that are seen to be restricted to certain dermatomes on careful examination, and asymptomatic corneal opacification with whorl-like cataracts on slit-lamp examination. Sudden cardiac arrhythmias, stroke, and renal failure are the most common causes of death in patients with Fabry's disease. In men with the classical form of the condition and in the absence of specific or supportive treatment, death occurs at a median age of 48 to 49 years, with a greatly reduced quality of life during the antecedent symp- tomatic period. Life expectancy in affected heterozygous women is also shortened. Sometimes the lancinating acroparaesthesias are sufficient to cause severe depression and even suicide. Diagnosis Diagnosis is made by demonstrating the abnormal glycolipid in urine or plasma, as well as by assay of α-galactosidase A in tears, plasma, white cells, dry blood spots, or other tissue material.

12.8 Lysosomal disease 2145 (a) (b) (c) (d) (g) (e) (f) (h) (i) Fig. 12.8.9 Facial images of nine men with the classical form of Fabry disease; although subtle, the facial appearances include periorbital oedema, depressed nasal bridge, prominent brow ridge, and full lips.

section 12 Metabolic disorders 2146 Molecular analysis of the α-galactosidase A gene on the long arm of the X chromosome is worthwhile because it allows the unambiguous detection of female heterozygotes and may thus be useful during the reproductive period, particularly for antenatal diagnosis. Despite the presence of active disease, ceramide trihexoside concentrations and α-galactosidase A assays are often within normal limits in af- fected female heterozygotes. Treatment Supportive care Hitherto, the treatment for Fabry's disease has been palliative, involving the use of anticonvulsants (including gabapentin) for the acroparaesthesias and neuropathic pain. Gastrointestinal symptoms sometimes respond to antimotility agents or to pancreatic enzyme supplements, but these agents have not been subjected to controlled trials. Renal failure is managed by dialysis or by renal transplant- ation; occasionally, cardiac transplantation has been required for cardiomyopathy; pacemakers and antiarrhythmic drugs may also be needed. Specific therapies To date, two preparations of recombinant human α-galactosidase A have been licensed: agalsidase-alfa (Replagal—not approved in the United States of America) and agalsidase-beta (Fabrazyme). These may differ slightly in their post-translational glycosylation status for delivery to endothelial, epithelial, and other cells that represent the pathological focus of this disease. Administration of these prepar- ations to male hemizygotes has improved lipid accumulation in the plasma and in renal biopsy samples. Both products have also been shown in double-blind, placebo-controlled trials to improve clinical endpoints of the disease, including neuropathic pain, stabilization of renal function, and ventricular mass, as well as conduction defects that represent infiltrative cardiomyopathy, but substantial reversal of established organ

malfunction has not been achieved. Unlike Gaucher’s disease, targeting to the affected cells and tis- sues in Fabry’s disease probably results from receptor-mediated uptake of protein molecules harbouring the common lysosomal recognition marker, mannose 6-phosphate, a less efficient and less specifically targeted system. In one remarkable instance, therapy with galactose infusions appears to have mitigated this condition by stabilizing the nascent mutant enzyme, thereby enhancing residual α-galactosidase A activity with slow clearance of cardiac glycolipid storage. Clinical trials of the pharmacological chaperone1- deoxyglactonojirimycin, migalastat, which is predicted to stabilize certain residual α-galactosidase A variants in Fabry’s disease and, by preventing misfolding, increase their delivery to the lysosome, have been reported. In patients naïve to therapy, who were found to have mutations amenable to the chaperone effect, statistically significant albeit modest reductions in the storage material were seen on histological analysis of kidney biopsy samples. In patients already on enzyme therapy, a further trial demonstrated that no additional decline in renal filtration function took place in the group randomized to migalastat. In both trials, reductions in left ventricular mass index were observed, although the full therapeutic meaning and clin- ical impact of this finding needs to be established. Migalastat has received marketing approval in the United States of America and Europe. Because of its distinct mechanism of action, which requires the binding of this inhibitory molecule to the active site of the en- zyme to achieve better folding, the drug is given in an alternate-day regimen to permit disengagement of the inhibitor from the enzyme once it has reached the lysosome. (a) (b) Fig. 12.8.10 Two patients with Fabry disease showing (a) diffuse telangiectatic lesions over flank and abdomen, and (b) hemispheric papules in suprapubic area. Reproduced with permission from Mulliken J. Capillary Malformations, Hyperkeratotic Stains, Telangiectasias, and Miscellaneous Vascular Blots. From: Mulliken and Young's Vascular Anomalies: Hemangiomas and Malformations, 2nd edition. Ed. John B. Mulliken, Patricia E. Burrows, and Steven J. Fishman. 2013. Courtesy of Dr Harley A. Haynes.

12.8 Lysosomal disease 2147 Mucopolysaccharidoses These disorders are caused by a deficiency of lysosomal hydrolases that catalyse the cleavage of complex glycosaminoglycans, which are macromolecular components of connective tissues including joints, bones, heart, and major arteries. Clinical manifestations of each of these disorders reflect an individual enzymatic deficiency and the resulting accumulation of mucopolysaccharide derivatives, of which dermatan-, keratan-, chondroitin-, and heparan sulphates are the principal components. In general, the accumulation of the complex substrates that are normally linked to proteins to form proteoglycans is associated with visceral enlargement, heart valve disease—as well as bony abnormalities, joint stiffness, corneal clouding and short stature. The accumulation of heparan sulphate may particularly be associated with the development of brain disease, including thick- ening of the leptomeninges, hence hydrocephalus is an often neg- lected factor in cerebral impairment that may also be attributed to lysosomal storage affecting neurons of the brain and peripheral gan- glia as well as the retina. Clinical features and pathology Typically, these disorders are associated with coarse facial features (Figs. 12.8.11 and 12.8.12), bone shortening, and skeletal abnor- malities, as well as disturbances of dentition, the gums, and middle ear. Abnormalities of the tracheobronchial cartilages and upper air- ways may be associated with respiratory infections and obstructive lung disease. The coronary arteries and cardiac valves may be infil- trated by glycosaminoglycans, leading to coronary artery occlusion and aortic and mitral valve malfunction. Similar changes may occur in peripheral arteries, particularly those supplying the viscera. In the eye, the basal layers of the cornea show swelling, cytoplasmic vacuolization, and storage granules leading to opacification. Scleral thickening may impinge upon the optic nerve. Excess urinary excretion of

glycosaminoglycan products, including dermatan sulphate and heparan sulphate, character- istically occur in the mucopolysaccharidoses. This abnormality should immediately prompt further investigations by enzymatic and genetic studies in blood leucocytes and/or fibroblasts obtained from cultured skin biopsy samples. The inheritance pattern of the mucopolysaccharidoses is typical of autosomal recessive traits with the exception of Hunter's disease (MPS II, which is due to iduronate sulphatase deficiency) that maps to the X chromosome and is ex- pressed predominantly in boys and men. Female heterozygotes for Hunter's disease only very rarely show evidence of neurological im- pairment or connective tissue abnormalities. Treatment Palliative treatment is a very important aspect of the management of these diseases and should include the provision of multidisciplinary support for children and young adults with the accompanying devel- opmental disabilities. Sustained provision for the long-term man- agement of the condition in affected families is desirable. Surgical procedures Corneal transplantation may be required to improve vision where retinal degeneration is not dominant. Carpel tunnel syndrome with compression neuropathy of the median nerve is very common and, when indicated, surgical treatment is often beneficial. Particular care is required in patients with mucopolysaccharidoses such as Hurler's syndrome when surgical procedures under general anaesthetic are required for relief of hydrocephalus, myringotomy, hernia repair, relief of airways obstruction due to laryngeal disease, and corrective spinal or joint surgery. Infiltration of the soft tissues of the upper and lower airways, as well as the heart and cervical spine (which may include subluxation of the atlanto-occipital joint), is associated with high perioperative mortality. Tracheostomy may be required to avoid life-threatening complications of intubation, Fig. 12.8.11 Facial image of a male patient aged 20 years with MPS I (Hurler's syndrome) who underwent a bone marrow transplant at the age of 1 year; some coarsening of the facial appearance persists. Fig. 12.8.12 Twenty-year-old female patient with Morquio A syndrome (MPS IV) with marked skeletal deformity and growth retardation.

section 12 Metabolic disorders 2148 but complications may arise with general anaesthesia beyond that of difficulties with endotracheal intubation. Extensive preoperative assessment should therefore be conducted whenever an anaesthetic is required for any procedure, particularly to assess the stability of the atlantoaxial joint, the airway, and the presence of coronary artery disease (that may predispose to perioperative myocardial infarc- tion). Where possible, an anaesthetist experienced in the manage- ment of patients with MPS disorders should be consulted. Specific treatments Bone marrow transplantation Bone marrow transplantation using HLA-identical sibling and HLA-matched nonsibling donors has been investigated extensively in the mucopolysaccharidoses. Long-term clinical trials have confirmed the beneficial effects of successful transplantation with reversal of hepatosplenomegaly and obstructive airways disease. In some cases there is improved longevity, with a possible reduction also in the incidence of sec- ondary hydrocephalus. However, at present, transplantation does not cure the condition and is unable to reverse established brain injury and most of the crippling skeletal manifestations. If it is to be considered, bone marrow transplantation should therefore be carried out early in the course of these diseases. The therapeutic position of bone marrow and cord blood-derived stem cell therapy is most clearly established for Hurler's disease (the more severe variant of MPS I). Enzyme replacement therapies Enzyme replacement therapy has long been under investigation in MPS I (Hurler's syndrome, Hurler– Scheie syndrome, and Scheie's syndrome), which was one of the first of such disorders to be subjected to intensive laboratory study. In clinical trials, recombinant human α-l-iduronidase, now licensed as laronidase (Aldurazyme) given by weekly infusion intravenously, after 1 year clearly showed a reduction in lysosomal storage: liver volume decreased

and there was an improved rate of growth as well as improvement in the range of joint movements at sites character- istic of connective tissue infiltration in this condition. With a reduc- tion in the storage material in the upper airways, there was also an improvement in episodes of hypoventilation during sleep. After a few weeks of enzyme treatment, urinary glycosaminoglycans abnor- malities were corrected. Although many patients developed serum antibodies, only transient immune reactions, including urticaria, occurred during the infusions. Enzyme replacement therapies have received market authoriza- tion for patients suffering from MPS II (Hunter's syndrome with iduronate sulphatase deficiency) and MPS VI (Maroteaux–Lamy disease due to arylsulphatase B deficiency) following successful clinical trials. Enzyme therapy with elosulfase alfa for MPS IVA (Morquio A) has been evaluated in clinical trials: benefits are re- ported in clinical measures of endurance and lung function and the therapy has received marketing approval in the United States of America and Europe, although the complexities of funding delayed patient access in the United Kingdom. Favourable responses to en- zyme replacement therapy have also been reported in animal models of related disorders, including the cone-head mouse that represents a faithful model of MPS VII (Sly's disease), due to deficiency of acid β-glucuronidase. Following results of a clinical trial in the exception- ally rare MPSVII, vestronidase alfa (MEPSEVII) received marketing authorisation from the U.S. Food and Drug Administration in 2017. Questions still arise of how clinical benefits and an improved quality of life can be best assessed. However, encouraging results showing an improved quality of life, mobility, nutrition, and educa- tional achievements have already been documented in several MPS disorders in response to enzyme therapy, even where pre-existing developmental effects and mental retardation are established. Pompe's disease Glycogen storage disease type II, due to acid maltase deficiency, otherwise known as Pompe's disease, is an autosomal recessive dis- order of glycogen metabolism caused by deficient activity of lyso- somal acid maltase—α-glucosidase. Acid maltase deficiency was the first of the lysosomal storage diseases to be so characterized by H.-G. Hers, a colleague of de Duve. The disease occurs in many countries and ethnic groups. The prevalence of this disorder is one in about 150 000 and males and females are affected equally. Clinical features and pathology Pompe first reported infants with massive cardiac hypertrophy and skeletal weakness with hypotonia, enlargement of the tongue and liver, and a uniformly fatal outcome. Acid maltase releases glucose units from the carbohydrate storage macromolecule, glycogen, as well as from the disaccharide, maltose. The enzyme is profoundly deficient in infants with Pompe's disease and partial deficiencies in the enzyme, detectable in all cells, are responsible for later-onset forms in children, adolescents, and adults. No clear correlation be- tween the degree of enzyme deficiency and the severity of disease is possible. Pathological accumulation of glycogen within vacuolar lyso- somal spaces occurs in skeletal muscles and (on occasion) other tissues, but it is noteworthy that in certain muscles, microscopic examination may be normal or show only trivial abnormalities, especially in patients with late-onset disease. Hence, the diagnosis of late-onset Pompe's disease may be difficult and routine muscle biopsies may not identify all those affected. The combined use of muscle biopsy with biochemical assays and molecular analysis of the acid glucosidase gene (at least for the common IVS1 mutation) should be considered in patients with unclassified myopathy. Acid maltase is normally responsible for constitutive autophagy and mo- lecular remodelling of intracytoplasmic glycogen; when deficient, abnormal glycogen accumulates within the lysosomal vacuole and elsewhere in the cell. In pathways not yet completely understood, this pathological accumulation is associated with tissue injury, but large cytoplasmic collections of autophagic debris appear to disrupt the contractile apparatus. Since glycogen is a storage molecule abun- dant in muscle cells, it is these cells that are the principal focus of acid maltase deficiency. Patients with infantile onset

of symptoms have predomin- antly skeletal muscle disease, hypertrophic cardiomyopathy, or macroglossia; hepatomegaly is not a feature of Pompe's disease in the absence of cardiac failure. Onset of disease in children and adults with weakness and poor athletic performance is associated with de- layed achievement of developmental motor milestones. Ultimately the clinical appearance is dominated by proximal muscle weakness with lordosis of the spine; patients adopt the Gower manoeuvre in rising from the squatting position. Late-onset forms observed in adults usually present as a progres- sive proximal myopathy with the variable addition of diaphragmatic

 and respiratory muscle paralysis leading to respiratory failure, but the rate of progression is unpredictable. The onset of symptoms varies between the age of 10 and 60 years. In most patients there is a history of longstanding proximal weakness with involvement of the truncal muscles and weakness in the hips in advance of the upper limb girdle. Poor physical strength and failure in gymnastic activities may be the clue. In children and adolescents, the condition may be misdiagnosed as a late-onset muscular dystrophy or even polymyositis, leading to inappropriate treatment. Ultimately the progressive proximal weakness is apparent and associated with re- spiratory failure: the latter is presaged by fatigue, breathlessness on exertion, and sleepiness due to marked ventilatory failure—carbon dioxide retention causes morning headaches. Occasionally, dys- phagia for solids may result from weakness of voluntary pharyngeal muscles that initiate swallowing. Treatment Alglucosidase alfa (Myozyme) has been developed as a mannose 6-phosphate-containing recombinant human acid α-glucosidase (rhGAA) for the treatment of patients of any age with Pompe's dis- ease (GSD II). Enzyme replacement therapy is administered to re- store enzymatic activity, deplete accumulated glycogen, and prevent its further accumulation to allow repair of damaged myocytes. In the very severe infantile form of the condition, where survival beyond 1 year of age is unusual, treatment with Myozyme has been associated with prolonged survival. In a trial of rhGAA in infants aged 6 months or younger, all were alive at 18 months whereas only 2% of the historical cohort group survived to this age. Most patients treated with rhGAA had normal growth and significant motor de- velopment during the treatment period. In another report, two se- verely affected (wheelchair- and ventilator-dependent) patients remained stable during an 8-year period of enzyme therapy, and in a third, moderately affected patient, muscle strength improved mark- edly and the ability to walk was regained. In some instances, however, the outcome has been disappointing and, in general, better outcomes are seen with early treatment and in patients who do not develop high-titre antibody responses to the re- combinant protein. Recently, in infants predicted to form high-titre, neutralising antibody responses on the basis of mutation analysis, immune-modifying treatments have been given at the outset of en- zyme therapy in an attempt to tolerize the immune system. Taken as a whole, the efficacy of enzyme replacement therapy for acid maltase deficiency emphasizes the need for prompt clinical recognition and diagnosis, especially in infants and young children. Several studies have confirmed the therapeutic efficacy of rhGAA in patients suffering from attenuated forms of Pompe's disease. In the present authors' experience with adult patients suffering from acid maltase deficiency, improvements in skeletal and respiratory muscle function are seen in the first year of treatment, with stability or a slower rate of decline maintained thereafter. It is unclear if, once lost, diaphragmatic function can be regained; we contend that re- stricting treatment to those with severely weak and wasted limbs and respiratory failure due to diaphragmatic paralysis will greatly underestimate its capacity to improve life quality or restore the func- tion of injured muscles. A second-generation enzyme preparation is undergoing clinical trial evaluation with the aim of improving the delivery of enzyme to the lysosomal compartment of skeletal muscle. The

Genzyme (Sanofi) agent is a form of α-glucosidase modified chemically to display many times more mannose phosphate than the parent com- pound, Myozyme. Even with specific treatment, the role of physical therapy, respira- tory assessment and support, nutritional care, and measures aimed at general rehabilitation remain crucial for functional outcome and improved quality of life.

Niemann–Pick diseases

Niemann–Pick disease types A and B

Niemann–Pick disease types A and B are, respectively, neuronopathic and non-neuronopathic variants of acid sphingomyelinase de- ficiency, a sphingolipid disorder leading to the accumulation of sphingomyelin. The condition resembles many of the manifest- ations of Gaucher's disease, with a characteristic secondary storage cell which is also a macrophage. The Niemann–Pick cell has a foamy appearance rather than the characteristic striated cytoplasm of the Gaucher cell: there is prominent infiltration of the lungs as well as the marrow cavity, liver and spleen. Niemann–Pick disease type A is associated with disabling neuronopathic features and dementia in infants and young chil- dren. Niemann–Pick disease type B may occur in adults who have only trivial splenomegaly and minor pulmonary infiltrates that are only exacerbated at times of intercurrent chest infection; they are at risk from osseous disease related to marrow infiltration, as with Gaucher's disease. At present, no specific treatments are available apart from the prompt treatment of pulmonary infection and the management of the consequences of skeletal infiltrates and episodes of avascular ne- crosis. Some patients, including those previously misdiagnosed as having Gaucher's disease, may have undergone splenectomy to relieve pressure symptoms or the haematological effects of hypersplenism. Since this disease is primarily a disorder of macrophages, it should be susceptible to enzymatic complementation using the mannose receptor. At the time of writing, clinical research to develop recom- binant human acid sphingomyelinase is well advanced. Clinical trials were delayed to address safety concerns from studies in animal models in which high-dose therapy led to a fatal inflammatory reac- tion, believed to be due to release of bioactive ceramide, the product of the catalytic reaction. After the completion of a gradual dose escalation study to min- imize release of ceramide product from accumulated substrate, a trial in adult patients with Niemann Pick type B disease is now continuing as an open-label phase II/III clinical trial to evaluate the safety and efficacy of different doses of recombinant acid sphingomyelinase when administered once every 2 weeks. In children up to the age of 18 years with this condition, a one-year phase I and II multicentre, open-label clinical trial to evaluate the safety and tolerability of recombinant human acid sphingomyelinase administered paren- terally once every 2 weeks is recruiting patients. It is intended that this agent will be started at 0.3 mg/kg dose, gradually increasing to a maximum of 3 mg/kg. The outcome of these long-awaited studies to advance the understanding of investigational enzyme therapy will be received with great interest in this very rare but severe dis- ease. Unfortunately, since the biosynthesis of sphingomyelin is not regulated by the uridine diphosphate-glucosylceramide synthase

 reaction, and so far no clinical inhibitors of this biosynthetic step are available, any exploration of substrate reduction therapy for the severe neuronopathic manifestations of Niemann–Pick disease type A will be long in coming.

Niemann–Pick disease type C

Niemann–Pick disease type C is a distinct disease that may pre- sent with jaundice in infants or children; the initial hepatitic illness usually resolves but may lead to fatal liver failure with cholestatic features. Intractable and progressive neurological disease occurs in childhood and early adult life, with ataxia, seizures, (vertical) supra- nuclear gaze palsy, and progressive diffuse cortical injury. Death usually occurs in the third or fourth decade. Niemann–Pick disease type C is not due to a primary defect of acid sphingomyelinase but to mutations in two distinct lysosomal proteins,

NPC1 and NPC2, that when mutated produce subtypes of the disease. The physiological role of the NPC1 transmembrane pro- tein remains unclear, as is the pathological cellular cascade that leads to NPC disease. Some investigators propose a lipid transport role for NPC1, others a role in lipid-sensing, while some evidence points to a complex involvement in endosomal calcium flux and other sec- ondary and downstream effects. Niemann–Pick disease type C is also associated with the appear- ance of foam cells in the macrophages; the Kupffer cells of the liver may be enlarged and a cholesterol trafficking defect is apparent in most cells. A rare complication is inflammatory bowel disease which has many features in common with Crohn's disease and a prominent infiltrate of storage macrophages in the inflamed tissue. The molecular defect in this disease, though not manifest in the skin, may be detected in skin-derived fibroblasts after culture and ex- posure to low-density lipoprotein cholesterol: in Niemann–Pick dis- ease type C, cholesterol is taken up and accumulates in intracellular droplets that stain positively with the fluorescent dye filipin. Within the brain, Niemann–Pick disease type C causes neuronophagia and the accumulation of gangliosides and other complex sphingolipid storage products that may induce neuronal injury. The use of statins and other agents that interfere with cholesterol metabolism has not been effective in arresting the course of this cruel illness. Clinical trials using N-butyldeoxynojirimycin (miglustat, Zavesca) have followed the delayed onset and increased survival of mice homozygous for a spontaneous mutation in the NPC1 gene that serves as an authentic model, recapitulating many features of the human disease. A randomized controlled trial and several co- hort studies have reported improvements in or stabilization of sac- cadic eye movements during 1 to 5 years of therapy. Swallowing was also shown to improve or remain stable during the randomized trial (up to 2 years). These findings were supported by long-term obser- vational cohorts (up to 6 years).
A meta-analysis of dysphagia—a clinically important therapeutic endpoint for the disease since as-piration pneumonia is a frequent cause of hospitalization and death—demonstrated a clear survival benefit with miglustat that was accompanied by improved swallowing. Serial studies showed decrease in calbindin in cerebrospinal fluid during treatment, sug- gesting reduced cerebellar Purkinje cell loss, and MRI studies dem- onstrated a protective effect on cerebellar and subcortical structure that correlated with clinical symptom severity. This research led to marketing approval of the drug by the European Medicines Agency, but it has yet to be approved for use in Niemann–Pick disease type C by the FDA in the United States of America. Treatment of Niemann– Pick C disease is currently the principal use of this agent, rather than type 1 Gaucher's disease, the original indication. Recently, two candidate products for preclinical development in Niemann–Pick type C have been identified. One is the recom- binant human heat-shock protein HSP70, and the other is an orally available small molecule, arimoclomol, that serves to induce heat- shock proteins, including HSP70. These candidates have produced encouraging biochemical and disease-modifying effects in the Niemann–Pick type C mouse model through their actions on lyso- somal integrity and on scrambled or denatured endogenous mol- ecules. The compounds have completed toxicity studies and clinical trial results are awaited. Cyclodextrins are complex ring structures that can solubilize lipids and are widely used domestic chemicals. When given system- ically and into the central nervous system, cyclodextrin was associ- ated with slowing and/or prevention of neurodegeneration in both a mouse and feline model of the disease. A multicentre phase III clinical trial of the intrathecal use (via lumbar puncture) of VTS- 270 (2-hydroxypropyl-β-cyclodextrin) is underway, as is a phase I/ IIa trial of its use in patients with neonatal hepatitis. Cyclodextrin infusions, especially via intrathecal administration, are a laborious and challenging intervention and it remains unclear how paren- teral cyclodextrin will find its therapeutic position in the long-term management of this disease, but orally active analogues are being explored for later application in

this disease. Cholesteryl ester storage disease and Wolman's disease These are late-onset (cholesteryl ester storage disease) and infantile (Wolman's disease) forms of lysosomal acid lipase deficiency, which causes the accumulation of cholesteryl esters in the lysosome. Wolman's disease is a devastating, fatal illness in which the infant fails to thrive, has massive hepatomegaly, adrenal calcification, and intestinal malabsorption. Death is almost inevitable within the first year of life. Cholesteryl ester storage disease, by contrast, manifests as a more indolent liver disease, with hepatic steatosis, progressing in many cases to fibrosis and cirrhosis. Patients have accelerated and often severe atherosclerosis with dyslipidaemia characterized by low plasma high-density lipoprotein cholesterol and variably high low- density lipoprotein cholesterol concentrations. It can be difficult to distinguish cholesterol ester storage disease from other commoner causes of fatty liver disease, except that the usual risk factors for the latter (overweight, diabetes, and other features of the metabolic syndrome) are normally absent and on histological analysis of liver biopsy specimens the lipid droplets are small, indicating lysosomal rather than cytosolic location: microvesicular steatosis is thus a hall- mark of cholesterol ester storage disease and macrovesicular stea- tosis reflects the spectrum of nonalcoholic liver disease. An enzyme preparation, sebelipase alfa, has been subject to in- tensive clinical trials for these disorders. The phase I clinical study demonstrated clear and early pharmacodynamic effects in patients with cholesteryl ester storage disease. Favourable outcomes of an international, randomized, double-blind, placebo-controlled phase III trial of sebelipase alfa in children and adults with lysosomal acid lipase deficiency, and the phase II/III trial of sebelipase alfa in infants

12.8 Lysosomal disease 2151 with Wolman's disease were reported in 2014. In summary, in 66 chil- dren and adults with lysosomal acid lipase deficiency administration of the enzyme met the primary endpoint of restoring of serum alanine aminotransferase (used as a biomarker of liver injury) to the healthy reference range. The agent was administered parenterally on alternate weeks at 1 mg/kg for the double-blind treatment period of 20 weeks. The median age of patients enrolled in the trial was 13 years of age (range 4–58) and fibrosis or cirrhosis was documented in all 32 pa- tients who had had baseline liver biopsy samples. In a continuing open- label follow-up study, relative to placebo, markers of dyslipidaemia and liver fat content improved, with sustained reduction in markers of liver injury and further improvements in low-density lipoprotein cholesterol. Worldwide marketing approval has been granted on the basis of these results. At the time of writing, funding for this treat- ment is available in some countries and is under negotiation in others (including the United Kingdom). There is evidence that sustained use of sebelipase may lead to a substantial reversal of fibrosis in this condi- tion and in the authors' view, the life-saving effects in children make a compelling case for authorized reimbursement. Danon's disease In 1981, two cases of cardiomyopathy in male infants with skeletal myopathy and mental retardation were reported by Danon and col- leagues. The skeletal pathology suggested type II glycogenosis but no deficiency of acid maltase activity was present. Mutations in the gene encoding LAMP2, located on the X-chromosome, have been iden- tified. LAMP2 is a highly glycosylated integral membrane protein of the lysosome with a role in mediating fusion of the autophagic vacuole with the lysosome. Deficiency leads to accumulation of vacuoles containing autophagic debris, including mitochondria and granular deposits of glycogen. In affected males, the clinical features include a dramatic hyper- trophic cardiomyopathy, a mild skeletal myopathy, and mild to mod- erate learning difficulties. The cardiomyopathy is particularly prone to give rise to malignant ventricular arrhythmias. Before the intro- duction of implanted defibrillation devices, the median age of death of classically affected hemizygotes was about 20 years. A milder phenotype, apparently restricted

to the heart, is seen in heterozy- gous women. Apart from supportive measures, no specific treatment is currently available, although a few hemizygous male patients have been successfully treated by cardiac transplantation. Mutations in the LAMP2 gene have been found at high frequency (6%) in men with unexplained severe hypertrophic cardiomyopathy. Diseases recently attributed to lysosomal dysfunction The characterization of lysosomal defects in several ill-understood disorders with diverse clinical manifestations continues to reveal much about the role of the lysosome in cellular functions of sig- nificance in medicine and molecular physiology. Several recently studied lysosomal diseases in this category are briefly described here. Neuronal ceroid lipofuscinoses Clinical features, genetic basis, and pathology The neuronal ceroid lipofuscinoses are the most common group of progressive brain diseases that usually affect children and young adults; 13 independent genetic groups have so far been identified with an estimated incidence of 1 in 12,500 live births. Ceroid lipofuscinosis, neuronal type 1 (CLN1) is due to mutations in a gene encoding palmitoyl:protein thioesterase 1, an enzyme in- volved in lysosomal degradation of acetylated proteins. CLN2 is due to defects in the gene encoding the acid hydrolase, tripeptidyl- peptidase. CLN3 is the most frequent form and particularly common in Nordic countries; it was the first lysosomal disease ever to have been reported in the literature (in a Norwegian family) in 1826, and is due to deficiency of a lysosomal transmembrane protein that may serve as a transporter molecule. Childhood forms of these disorders are almost invariably inherited as recessive traits and result in a progressive dementia combined with epilepsy (sometimes myoclonic), blindness, and early death. The family history may, however, suggest dominant transmission of CLN11, a puzzling recessive disease caused by mutations in the GRN gene encoding progranulin, with confusion arising because hetero- zygotes develop frontotemporal lobar degeneration with ubiquitin- positive inclusions (Online Mendelian Inheritance in Man (OMIM) 607485). In only one of these conditions, Kufs adult-onset neuronal lipofuscinosis, is inheritance of a single copy of the mutant CLN4/ DNAJC5 gene both necessary and sufficient to cause disease, which is always transmitted as an autosomal dominant. In several instances, neuronal ceroid lipofuscinoses represents defects in elements of intralysosomal protein catabolism, indi- cating that the turnover of the cognate proteins is very high in cor- tical neurons. Realization that the neuronal ceroid lipofuscinoses represent inherited disorders of lysosomal protein metabolism is very recent, but the discovery clearly has important consequences for better understanding the pathology of this family of cruel neurodegenerative disorders and for developing better diagnostic tools (especially for prenatal application) as well as innovative treatments The most familiar form of these diseases has, in Anglophone coun- tries, been widely termed ‘Batten’s disease’. In 1915, Dr F.E. Batten had, at a time when these disorders fell into the descriptive category of ‘familial amaurotic idiocy’, correctly differentiated infantile neur- onal ceroid lipofuscinosis from Tay–Sachs disease. However, with identification of the biochemical causation and responsible genetic loci, this terminology and many other eponymous terms from the medical literature of the 19th and 20th centuries now has little prac- tical value in the enlarging canon of lysosomal disorders. The striking pleiotropic effects of mutations at loci responsible for the neuronal ceroid lipofuscinoses reflect the severe impairments of multiple lysosomal functions which cause this class of exclusively neurodegenerative diseases. Several of the genes encode lysosomal proteins, including acid hydrolases (CLN1, CLN2, CLN10, CLN13); a soluble lysosomal protein in CLN11; a protein, progranulin, that functions in the secretory pathway; two cytoplasmic proteins that interact with lysosomal membranes (CLN4, CLN14); and many transmembrane proteins with diverse subcellular locations (CLN3, CLN6, CLN7, CLN8—and the lysosomal ATPase, type 13A2 in CLN12). Pathological studies show the characteristic accumulation of autofluorescent storage debris (lipofuscin) within

neurons and lysosomes in other cells; this material consists of several oxidized and ubiquitinated proteins and often includes soluble cytochrome C derived from the mitochondrial F1ATPase complex and saposin fragments. The storage of this material occurs preferentially in

section 12 Metabolic disorders 2152 lysosomes of the nervous system and is associated with progressive neuronal death leading to a marked atrophy of the brain; cerebral atrophy is particularly obvious in the early-onset forms of the neur- onal lipofuscinoses. Diagnosis Diagnosis of these diseases requires clinical persistence, which is driven by the need for clarity and to provide genetic and prognostic advice to family members and carers. The electroencephalogram is usually informative with early development of occipital spike poten- tials after photic stimulation. MRI shows atrophy, characteristically first in the cerebellum and vermis but which progresses to general- ized cerebral atrophy, ultimately with profound shrinkage (unlike GM2 gangliosidosis from which the CLN syndromes need to be distinguished). The presence on a blood smear of vacuoles in lymphocytes in a juvenile disorder would be typical of CLN3 disease. Enzymatic tests conducted on white cell pellets can readily define suspected CLN1 or CLN2 disease. Ultrastructural studies by electron microscopy in blood cells and fibroblasts may demonstrate the characteristic storage deposits, usually in lysosomal structures, in blood cells or tissue specimens. Advances in molecular diagnostics allow the iden- tification of defective genes and their protein products in several dis- tinct clinical phenotypes. Supportive and symptomatic management This clinically heterogeneous family of relentless neurodegenerative diseases poses great challenges: the provision of continuing care for what in most cases is a chronic, cruel, and fatal disease affecting chil- dren and young adults. As described, neuronal ceroid lipofuscinoses are characterized by dementia (one of the most frequent causes in young persons), epilepsy, motor deterioration, and visual loss, and for most there is currently no specific therapy and little prospect of therapy. Discovery of the genetic basis of 14 clinical variants permits prenatal and postnatal diagnosis in affected pedigrees by molecular analysis of genomic DNA that is of key importance for provision of genetic counselling. Beyond an experimental therapy, and one approved molecular therapy, palliative measures are employed for symptom relief. Much distress accompanies loss of the ability to swallow and vocalize and of independent movement. While the use of feeding gastrostomies is critical for maintaining hydration and giving regular medication to treat epilepsy and muscle spasms, death is usually inevitable in CLN2 by the early-mid teenage years, often from aspiration pneumonia. The response to anticonvulsant medication is best judged by the level of symptomatic relief and the minimum effective dose for rea- sonable clinical control should be used. Complex drug regimens, especially those that use more than two drugs, often compound the unwanted effects and are counterproductive. Sodium valproate and lamotrigine are preferred, and phenytoin, carbamazepine, and vigabatrin (and topiramate in CLN2 disease) are probably best avoided in patients with neuronal ceroid lipofuscinoses. In generalized persistent seizures, diazepam and/or lorazepam are used in the short term to gain control. Under these circum- stances there may be a place for introducing phenobarbitone for frequent severe attacks, and myoclonic seizures may also respond to ethosuximide. Another valuable measure, when frequent severe attacks occur, is the introduction of a ketogenic diet, which is often of decisive benefit in patients whose seizures are otherwise very challenging. Myoclonus may be exacerbated by carbamazepine and gabapentin (and pregabalin) as well lamotrigine, hence careful reduction of the anticonvulsant regimen may help to control this often distressing manifestation. Zonisamide has been reported to control myoclonus, and levetiracetam and piracetam may also have therapeutic effects. Spasticity requires prompt and enthusiastic use of physiotherapy and sometimes splinting to prevent or mitigate the

tendency for development of fixed flexural deformities with painful spasms and emergence of pressure sores. Local use of botulinum toxin may as- sist in severe cases. The spasmolytic agents baclofen and tizanidine can be effective, and there are preliminary reports that tetrahydro-cannabinol may be useful in some patients. Benzodiazepines are no longer popular since their unwanted effects (excessive drowsiness and dribbling) are so unwelcome. Experimental and specific therapies Cysteamine In vitro studies suggested that the use of the lysosomotropic thiol agent, cysteamine, may activate residual palmitoyl-protein thioesterase activity in patients with CLN1 or solubilize the intralysosomal ceroid in this disease. Cysteamine bitartrate (Cystagon), used in the treatment of cystinosis, was ex- plored in a 7-year, open-label, substrate-reduction therapy trial in four patients with atypical, juvenile-onset CLN1. Five untreated pa- tients with the same CLN1 mutations, three of whom were siblings, were included as controls. The treatment substantially decreased the storage material in peripheral lymphocytes in a dose-dependent manner, and a minor slowing of the disease progression compared to the controls was observed in three out of the four treated patients. A long-term pilot trial of oral cysteamine bitartrate and N-acetylcysteine was conducted in 10 children below 3 years of age with any two of the seven most lethal CLN1 mutations. Outcomes in nine patients after follow-up for 8 to 75 months were compared with the reported natural history of the disease and that of affected older siblings. While no trial participant acquired new skills and retinal function decreased progressively, the average time to an isoelectric EEG (52 months) was longer than in historical controls (36 months), and parents and physicians reported less irritability, improved alert- ness, or both in seven patients. It seems unlikely that this treatment will be used widely. Enzymatic augmentation and gene therapy in CLN2 Recombinant human tripeptidyl peptidase 1 was investigated as a potential treat- ment for CLN2 disease in children aged 3 to 16 in an open-label, multicentre clinical trial. The protein, termed cerliponase-alfa, at 30, 100, and 300 mg was initially infused at 2-weekly intervals into cerebral ventricles through an in-dwelling device, later maintained at 300 mg over at least 96 weeks. Outcome was determined by de- cline in a motor-language disease score, with data compared with a study conducted on the course of the disease in historical controls. The mean (± standard deviation) unadjusted rate of decline in the motor-language score per 48-week period was 0.27 ± 0.35 points in treated patients and 2.12 ± 0.98 points in 42 historical controls (mean difference, 1.85). Cerliponase alfa received global marketing approval as Brienura from the FDA (for children >3 years) and European Medicines Agency in 2017 for patients at any age. At the time of writing in the United Kingdom, NICE has given provisional

 approval for Brienura with limited reimbursement from the National Health Service in England under a managed access programme. Anecdotal reports and post-marketing information provides some reassurance that this intensive and laborious intervention provides clinically useful benefit and stabilization of disease in otherwise stricken children. It is not yet known whether the therapy will delay the onset of blindness. Two small-scale early-phase clinical trials of gene therapy have been conducted in infants with CLN2 using recombinant adeno-associated vectors delivered intracranially. Two distinct vector serotypes with potentially different cellular tropisms have been used. At the time of writing no beneficial outcomes have been reported from either trial. Papillon–Lefèvre syndrome This is an unusual syndrome, inherited as an autosomal reces- sive trait, resulting in periodontal disease with tooth loss and palmoplantar keratosis that is associated with a selective deficiency of cathepsin C activity within the azurophil granules of neutrophilic polymorphonuclear leucocytes. Several mutations have been identi- fied within the gene encoding cathepsin C, which is an exo-cysteine protease, also known as dipeptidyl

peptidase I, that serves as a multi- faceted scaffold on which numerous chymotrypsin-like proteases are activated during neutrophil maturation. These include granule serine peptidases such as elastase, cathepsin G and proteinase 3 in neutrophils, and chymase and tryptase in mast cells; a partial role in the activation of granzyme B, a key effector system of natural killer cells, has been suspected from animal studies but only described in one affected human pedigree. Deficiency of antimicrobial pep- tides released during the normal inflammatory process has also been shown. A more severe allelic variant known as Haim–Munk syndrome, originally reported from Cochin in Southern India, is associated with onychogryphosis, pes planus, arachnodactyly, and osteolysis involving the distal phalanges (acro-osteolysis). It appears that the enzyme deficiency leads to the failure of bac- terial clearance in the gums, thereby causing destructive periodon- titis and tooth loss. The corresponding role of cathepsin C within the dermal epithelium is not known, but a failure of cathepsin C activity reproducibly leads to epithelial abnormalities and thickening of the skin, particularly on the soles of the feet. Some patients with disabling skin manifestations have obtained benefit by the use of retinoids, with or without antimicrobial therapy. These agents are, however, unlikely to improve early-onset destructive periodontal disease which leads to loss of primary and secondary dentition. Recurrent oral infection with Aggregatibacter actinomycetemcomitans infection has been reported. The importance of the Papillon–Lefèvre syndrome rests not only on the identification of lysosomal cathepsin C as an important com- ponent of immune defences against bacteria that preferentially in- vade the privileged periodontal site, but also on the involvement of this enzyme in the normal turnover of keratinized skin as well as defence against microbial invasion. Spondyloenchondrodysplasia with immune dysregulation This autosomal recessive skeletal dysplasia with intracranial calcifi- cation had been long recognized, but association with deficiency of the lysosomal tartrate-resistant iron-containing purple (type 5) acid phosphatase and diverse clinical manifestations of autoimmunity, including lupus erythematosus, has been recent. The type 5 acid phosphatase is a readily measured lysosomal en- zyme expressed in osteoclasts and pathological macrophages. In healthy persons, the enzyme is also abundant in Langerhans and dendritic cells. Apart from its ability to degrade skeletal phospho- proteins, including osteopontin, it probably modulates the effector pathways of phagocytic activation or antigen presentation. There is little or no evidence of ‘storage’: the disease illustrates the extraordinary diversity of lysosomal functions in the whole animal and how genetic disturbances can induce wide-ranging clinical ef- fects. Enchondromatous lesions are seen in long bones with sclerosis and irregularity of the metaphyseal plate. Lateral spine radiographs reveal platyspondyly and irregularity of the vertebral endplates. There is intracranial calcification in the basal ganglia, thalami and deep cerebral gyri. The manifestations of autoimmunity are characterized by elevated antinuclear antibody and anti-double-stranded DNA antibody titres with hypocomplementaemia. The clinical course in patients is varied but generally florid, with hypothyroidism, vitiligo, thrombocytopenia requiring splenectomy, autoimmune haemolytic anaemia, hepatosplenomegaly, nonerosive arthropathy, and vascu- litic skin eruptions. Defects of organelle assembly: Chédiak–Higashi, Griscelli’s, and Hermansky–Pudlak syndromes Inherited defects of protein complexes that participate in the bio-genesis of lysosomes and their related secretory organelles such as melanosomes are increasingly being recognized. The organ- elles with specialized functions that closely resemble lysosomes are termed lysosome-related organelles and include δ-granules in platelets; Weibel–Palade bodies of endothelial cells; lytic granules and vesicles implicated in the immune ‘synapse’ in lymphocytes; basophil and azurophil granules in polymorphonuclear leucocytes; lamellar bodies in type 2 pneumocytes; neuromelanin granules in the catecholaminergic neurones of the nigro-strial pathway, and the melanosomes of the iris, choroid, and skin. Most of these organelles maintain an

acidic intra-organellar milieu, but while they often em- ploy the lysosomal recognition marker, mannose 6-phosphate, they do not necessarily have a full complement of lysosomal membrane proteins such as LAMP1 and LAMP2 and other characteristics. Chédiak–Higashi, Griscelli's, and Hermansky–Pudlak syndromes are rare conditions inherited as autosomal recessive traits. All cause oculocutaneous albinism, often in association with abnormal platelet granules and melanosomes in the skin and eyes: partial al- binism is frequent. Chédiak–Higashi and Griscelli's syndromes Chédiak–Higashi syndrome is caused by mutations in the lyso- somal trafficking regulator gene located on chromosome 1q44. It predisposes to microbial infection and there are giant lysosomal granules in peripheral blood granulocytes; ceroid storage occurs in the nervous system and lungs. The clinical phenotype results from a complex set of immune defects affecting natural killer cells and neutrophilic leucocytes. Natural killer cell cytotoxicity is absent. Neutrophils, melanocytes, neurons, muscle cells, and Schwann cells show giant inclusion bodies. Recurrent cutaneous and systemic pyogenic infections occur with defective neutrophil and monocyte migration. Neurodegeneration is a prominent feature in young adults, but death often results from a rapidly progressive lymphoproliferative disorder.

section 12 Metabolic disorders 2154 The Griscelli's syndrome(s) are three unusual variants: one (type III) is a simple form of albinism, and the others combine albinism with defective immunity (type II) or neurological deficits (type I). Griscelli's syndrome type II with immunological defects is caused by mutations in Rab27a, a soluble GTPase, which regulates the flow of melanosomes in melanocytes and regulates exocytosis of lytic gran- ules at the point of the 'immune synapse' in cytotoxic T lympho- cytes. Deficient Rab27a thus causes dysfunctional T lymphocytes and pigmentary abnormalities. The Griscelli's syndrome type I, which also has neurological symptoms, is caused by mutations in the motor protein, myosin Va, which may cooperate with Rab27a to transport melanosomes along actin filaments but apparently does not participate in the exocytosis of lytic T cell granules. Hermansky–Pudlak syndrome Nine genetically distinct Hermansky–Pudlak disorders are known. Hermansky–Pudlak syndrome type 2 is caused by mutations in the β-3A adaptin gene which is associated with altered trafficking of lysosomal proteins in melanosomes, lysosomes, and platelet- dense granules leading to storage pool deficiency. The gene maps to chromosome 10q. Inheritance is autosomal recessive. Although very rare, one of the Hermansky–Pudlak syndromes occurs at a high frequency in the Swiss Alps and the Puerto-Rican population where it is the most common single-gene defect. Clinical features include a bleeding tendency due to abnormal platelets; diminished pigmentation of the skin, iris, and hair; and diverse inflammatory complications including granulomatous col- itis, cardiomyopathy, and severe pulmonary fibrosis. The pulmonary disease appears to be related to defective release of surfactant by type 2 pneumocytes. Hermansky–Pudlak syndrome type 2 causes a mild bleeding di- athesis and platelet dense bodies are absent; the patients are suscep- tible to bacterial infection due to congenital neutropenia. There are clear similarities between Hermansky–Pudlak and Chédiak–Higashi syndromes, and further functional studies of their respective cognate proteins should refine our knowledge about the regulation of synthesis and coordinated assembly of lysosomes and related organelles. Treatment of biogenesis defects These disorders are often very severe. For example, patients with Chédiak–Higashi disease and Griscelli's syndrome type 2 occasion- ally develop a life-threatening syndrome with fever, jaundice, and pancytopenia—haemophagocytic lymphohistiocytosis—which is related to impaired natural killer and cytotoxic T-cell function and failure to resolve of lymphocyte and macrophage activation. These cells proliferate, releasing inflammatory cytokines that in- duce fever. Jaundice,

hepatosplenomegaly, and pancytopenia are present in a hyperacute illness which is usually triggered by infec- tion with Epstein–Barr virus or other viruses. High-dose cortico- steroids and immunomodulatory agents are needed to suppress the inflammation. Rituximab and ciclosporin have been successful where the response to corticosteroids, ciclosporin, and etoposide was inadequate. When this accelerated lymphohistiocytic phase of the illness is resolved, transplantation with haematopoietic stem cells which replace the defective components of immune system with normal effector cells reduces the risk that this potentially fatal syndrome will recur. Oculocutaneous albinism may require aids for poor vision due to retinal photoinjury, especially at school, and protection against high-intensity ultraviolet and visible light-induced damage, with skin carcinoma, should be offered. Haemorrhagic manifestations may require platelet transfusions and parenteral desmopressin (DDAVP—1-desamino-8-d-arginine vasopressin) to improve platelet function in the short term. Aspirin and other nonsteroidal drugs should be avoided if possible. Serious microbial infections with bacteria are common in affected children and fungal infections due to Candida or Aspergillus also occur. Immunization for common viral and bacterial infections, including influenza, Haemophilus influenzae, and pneumococci, should be given, and appropriate antimicrobial drugs used promptly where infection is likely. Established pulmonary fibrosis proceeds rapidly and may require treatment with domiciliary supplemental oxygen in the home, and lung transplantation may be successful in selected cases. A smoke- free environment is likely to be advantageous. FURTHER READING The study of lysosomal diseases is burgeoning: critical cellular func- tions carried out in the lysosomal compartment place the study of this organelle at the heart of contemporary molecular cell biology. The sheer pace of discovery and involvement of the lysosome in many pathological conditions and processes, with or without an overt gen- etic basis, means that comprehensive sources of up-to-date infor- mation are hard to find. Here we principally identify references of immediate application in the clinical field. Books Alberts B, et al. (2007). Molecular biology of the cell, 5th edition. Garland Science (Taylor & Francis Group), New York. Barranger JA, Cabrera-Salazar MA (eds) (2007). Lysosomal storage dis- orders. Springer Science, New York. Mehta A, Winchester B (eds) (2012). Lysosomal storage diseases: a prac- tical guide. Wiley-Blackwell, London. Mole S, Williams R, Goebel H (eds) (2011). The neuronal ceroid lipo- fuscinoses (Batten disease), 2nd edition. Oxford University Press, Oxford. Nyhan WL, Barshop BA, Ozand PT (eds) (2005). Atlas of metabolic diseases, 2nd edition. Hodder Education, London. Saftig P (ed) (2005). Lysosomes. Springer Science, New York. Reviews of diagnosis and treatment of lysosomal diseases Anderson G, et al. (2005). Blood film examination for vacuolated lymphocytes in the diagnosis of metabolic disorders; retrospective experience of more than 2,500 cases from a single centre. J Clin Pathol, 58, 1305–10. Baldo BA (2015). Enzymes approved for human therapy: indications, mechanisms and adverse effects. BioDrugs, 29, 31–55. Cox TM (2016). Lysosomal diseases. In: Bond JD (ed) Encyclopedia of Cell Biology, Vol. I, pp. 763–88. Elsevier, Waltham. De Duve C (1964). From cytases to lysosomes. Fed Proc, 23, 1045–9. Hocquemiller M, et al. (2016). Adeno-associated virus-based gene therapy for CNS diseases. Hum Gene Ther, 27, 478–96. Kornfeld S, Mellman I (1989). The biogenesis of lysosomes. Annu Rev Cell Biol, 5, 483–525. Mindell JA (2012). Lysosomal acidification mechanisms. Annu Rev Physiol, 74, 69–86.

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