

# THE CELL BIOLOGY OF LYSOSOMAL STORAGE DISORDERS

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Lysosomal storage disorders, of which more than 40 are known, are caused by the defective activity of lysosomal proteins, which results in the intra-lysosomal accumulation of undegraded metabolites. Despite years of study of the genetic and molecular bases of lysosomal storage disorders, little is known about the events that lead from this intra-lysosomal accumulation to pathology. Here, we summarize the biochemistry of lysosomal storage disorders. We then discuss downstream cellular pathways that are potentially affected in these disorders and that might help us to delineate their pathological mechanisms.

The existence of a 'new group of particles with lytic properties' — the so-called 'suicide bags', which were subsequently named lysosomes — was discovered by de Duve and colleagues. Ever since, there has been great interest in their role in disease — in particular, in those diseases that result from the 'failure of lysosomal enzymes to function properly'<sup>1</sup>. Although we do not wish to argue with as distinguished a scientist as Christian de Duve, the comment in his [Nobel lecture](#) of 1974 (REF. 1) that, 'the mysterious chapter of the pathology of congenital lysosomal enzyme deficiencies has been largely elucidated' might have been a little optimistic. It is true — and this was probably the intent of de Duve — that a large number of metabolic deficiencies in lysosomal enzymes and integral membrane proteins have been described, and that the enzymatic, genetic and molecular bases have been determined for many of them. However, the scientific literature is strangely silent on the downstream or secondary biochemical and cellular pathways that are affected in these diseases, and that result in cell and tissue dysfunction and therefore pathology. In other words, why does the lysosomal accumulation of any particular unmetabolized substrate cause cell dysfunction, with little apparent correlation between the type of substrate that accumulates and the course of the disease?

Here, we attempt to address this question. First, we outline the main biochemical causes of lysosomal storage disorders (LSDs) and, in particular, highlight some

recent unexpected findings that shed new light on the biochemical basis of some well-established LSDs. We then discuss how the lysosomal accumulation of undigested metabolites might impact downstream pathways and cause pathology, and also attempt to convince readers that studying the cellular defects in LSDs provides a unique opportunity to gain insights into normal cell physiology, and not just pathophysiology.

The biochemistry of lysosomal storage disorders. The lytic particles that were first described by de Duve — the lysosomes — are membrane-bound organelles that consist of a limiting, external membrane and intra-lysosomal vesicles<sup>2</sup>. Lysosomes contain digestive enzymes that are active at the acidic pH of this organelle, and most of these enzymes are soluble and localized in the lysosomal lumen. Recent studies, including a proteomic analysis<sup>3</sup>, have identified new soluble lysosomal proteins, including hydrolases, and integral membrane proteins<sup>4</sup>. This has led to estimates that there are at least 50–60 soluble hydrolases<sup>3</sup> and at least 7 integral membrane proteins<sup>4</sup> in lysosomes. In principle, mutations in the genes that encode any of these proteins could cause an LSD. Over 40 LSDs that involve soluble hydrolases are known and, recently, a number of diseases have been identified that involve the integral membrane proteins (TABLE 1; for a more complete version of this table, please see online [supplementary information S1](#) (table)). Indeed, a genetic disorder will probably be discovered for every lysosomal

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Table 1 | Lysosomal storage disorders\*

Disease	Defective protein	Main storage materials
<b>Sphingolipidoses</b>		
Fabry	$\alpha$ -Galactosidase A	Globotriaosylceramide and blood-group-B substances
Farber lipogranulomatosis	Ceramidase	Ceramide
Gaucher	$\beta$ -Glucosidase Saposin-C activator	Glucosylceramide Glucosylceramide
Niemann–Pick A and B	Sphingomyelinase	Sphingomyelin
Sphingolipid-activator deficiency	Sphingolipid activator	Glycolipids
GM1 gangliosidosis	$\beta$ -Galactosidase	GM1 ganglioside
GM2 gangliosidosis (Tay–Sachs)	$\beta$ -Hexosaminidase A	GM2 ganglioside and related glycolipids
GM2 gangliosidosis (Sandhoff)	$\beta$ -Hexosaminidase A and B	GM2 ganglioside and related glycolipids
GM2 gangliosidosis (GM2-activator deficiency)	GM2-activator protein	GM2 ganglioside and related glycolipids
<b>Mucopolysaccharidoses (MPS)</b>		
MPS I (Hurler, Scheie, Hurler/Scheie)	$\alpha$ -Iduronidase	Dermatan sulphate and heparan sulphate
MPS II (Hunter)	Iduronate-2-sulphatase	Dermatan sulphate and heparan sulphate
MPS IIIA (Sanfilippo)	Heparan <i>N</i> -sulphatase (sulphamidase)	Heparan sulphate
MPS IIIB (Sanfilippo)	<i>N</i> -Acetyl- $\alpha$ -glucosaminidase	Heparan sulphate
MPS IIIC (Sanfilippo)	Acetyl-CoA: $\alpha$ -glucosamide <i>N</i> -acetyltransferase	Heparan sulphate
MPS IIID (Sanfilippo)	<i>N</i> -Acetylglucosamine-6-sulphatase	Heparan sulphate
Morquio-A disease	<i>N</i> -Acetylgalactosamine -6-sulphate-sulphatase	Keratan sulphate, chondroitin-6-sulphate
Morquio-B disease	$\beta$ -Galactosidase	Keratan sulphate
MPS VI (Maroteaux–Lamy)	<i>N</i> -Acetylgalactosamine-4-sulphatase (arylsulphatase B)	Dermatan sulphate
MPS VII (Sly)	$\beta$ -Glucuronidase	Heparan sulphate, dermatan sulphate, chondroitin-4- and -6-sulphates
<b>Oligosaccharidoses and glycoproteinosis</b>		
Pompe (glycogen-storage-disease type II)	$\alpha$ -Glucosidase	Glycogen
<b>Diseases caused by defects in integral membrane proteins</b>		
Cystinosis	Cystinosisin	Cystine
Danon disease	LAMP2	Cytoplasmic debris and glycogen
Infantile sialic-acid-storage disease and Salla disease	Sialin	Sialic acid
Mucopolipidosis (ML) IV	Mucopolipin-1	Lipids and acid mucopolysaccharides
Niemann–Pick C (NPC)	NPC1 and 2 <sup>†</sup>	Cholesterol and sphingolipids
<b>Others</b>		
Galactosialidosis	Cathepsin A	Sialyloligosaccharides
I Cell and pseudo-Hurler polydystrophy (ML II and ML III, respectively) <sup>§</sup>	UDP- <i>N</i> -acetylglucosamine:lysosomal enzyme <i>N</i> -acetylglucosaminyl-1- phosphotransferase	Oligosaccharides, mucopolysaccharides and lipids
Multiple sulphatase deficiency	C $\alpha$ -formylglycine-generating enzyme	Sulphatides
Neuronal ceroid lipofuscinosis (NCL)1 (Batten disease) <sup>  </sup>	CLN1 (protein palmitoylthioesterase-1)	Lipidated thioesters
NCL2 (Batten disease)	CLN2 (tripeptidyl amino peptidase-1)	Subunit c of the mitochondrial ATP synthase
NCL3 (Batten disease)	Arginine transporter	Subunit c of the mitochondrial ATP synthase

\*This table shows lysosomal storage disorders (LSDs) that are discussed in this article. For a complete list of LSDs, please see online supplementary information S1 (table). Detailed information about each disease can be obtained from **REF. 108**, and complementary lists of LSDs can be found in **REFS 89,109**. The prevalence of some of LSDs has been studied in a pan-ethnic Australian population<sup>110</sup>, which does not specifically address ethnic groups in which some of the diseases are more prevalent. <sup>†</sup>NPC1 might transport cholesterol. Alternatively, it might be involved in sphingolipid homeostasis<sup>53</sup>. The related NCP1-like protein-1 (NPC1L1), which contains a similar sterol-sensing domain to NPC1, is involved in cholesterol absorption in intestinal cells<sup>111</sup>. NPC1 and the cholesterol-binding NPC2 in the lysosome lumen might function together<sup>112</sup>. <sup>§</sup>ML II and ML III were originally classified as mucopolipidoses, but they are now known to be caused by the defective transport of lysosomal enzymes to lysosomes in cells of mesenchymal origin, rather than by a defect in lysosomal lipases. <sup>||</sup>There are at least eight classes of NCL<sup>16</sup>, but the function of the defective proteins is only known for NCL1, 2 and, possibly, 3 (**REF. 22**). LAMP2, lysosome-associated membrane protein-2.

## Box 1 | Disease symptoms in lysosomal storage disorders

Most lysosomal storage disorders (LSDs) exist in infantile, juvenile and adult forms. The most severe, infantile forms, present with acute brain involvement and patients die within the first years of life. In adult forms, symptoms develop more slowly and disability often arises mainly from peripheral symptoms. Juvenile forms are intermediate between infantile and adult forms. Neurological symptoms can include seizures, dementia and brainstem dysfunction. Among the peripheral symptoms are enlargement of the spleen and liver (hepatosplenomegaly), heart and kidney injury, abnormal bone formation, muscle atrophy and ocular disease. Several diseases are characterized by prominent neurological involvement and minimal peripheral impairment (for example, Sanfilippo disease), whereas others have peripheral dysfunction with rare brain involvement (for example, Fabry disease). Indeed, each LSD has a distinct clinical and pathological picture, which presumably relates somehow to the nature of the accumulating substrate and the cell types in which it accumulates. For example, all forms of Pompe disease (or glycogen-storage-disease type II) are characterized by myopathy (muscle pathology), which is expected on the basis of the important role that glycogen has in muscle. And many of the sphingolipidoses are characterized by brain disease, which might be expected on the basis of the high levels at which glycosphingolipids are found in the brain. However, only a small fraction of patients with Gaucher disease (a sphingolipidosis), for example, show neurological signs, and it is not clear why there are subclasses of this disease that have or do not have neurological involvement. In some diseases, phenotypic variability can be explained by differing levels of residual enzyme activity, but in others there is no obvious genotype–phenotype correlation and patients with a similar genetic background, and sometimes with the same mutation, can present with widely differing clinical symptoms, or even be asymptomatic for disease. This is presumably due to modifying genes and environmental factors that can influence the clinical course. Together, these observations indicate an important role for secondary metabolites or downstream cellular pathways in disease pathology.

#### MANNOSE-6-PHOSPHATE RECEPTORS

Receptors that are located at the *trans*-Golgi network and, at low levels, at the plasma membrane. They are responsible for targeting several soluble lysosomal hydrolases from the Golgi, through endosomes, to lysosomes.

#### SPHINGOMYELIN

A sphingolipid that contains a phosphorylcholine headgroup.

#### GLYCOSAMINOGLYCANS

Long, linear, charged polysaccharides that are composed of a repeating pair of sugars, of which one is an amino sugar.

#### SPHINGOLIPIDS

Membrane lipids containing a ceramide backbone. Ceramide consists of a long chain sphingoid base, to which a fatty acid is linked through the amino group at C2. A headgroup is linked to the hydroxyl at C1.

#### GANGLIOSIDE

A sialic-acid-containing glycosphingolipid that accumulates in the gangliosidoses.

protein eventually, although some might be difficult to identify due to the essential nature of the protein. For example, no LSD is known that involves the vacuolar-type H<sup>+</sup>-ATPase, which is responsible for maintaining the acid pH of lysosomes<sup>5</sup>. This is because an inability to maintain the acidic pH of lysosomes would lead to a greatly reduced activity of all the lysosomal hydrolases and to the accumulation of huge amounts of unmetabolized substrates, which would presumably be lethal at early stages of development.

LSDs can be grouped according to various classifications, but perhaps the most useful is based on characterization of the defective enzyme or protein, rather than on the nature of the accumulated substrate(s) (TABLE 1; see also online [supplementary information S1](#) (table)). The latter has been the cause of the erroneous characterization of a number of diseases for which the accumulating substrate was characterized years before the enzymatic defect was known. For example, I Cell disease (ML II) and pseudo-Hurler polydystrophy (ML III) — which were previously characterized as ‘mucopolysaccharidoses’ (MPS) — are now known to be caused by the defective transport of lysosomal enzymes to lysosomes through the MANNOSE-6-PHOSPHATE-RECEPTOR system rather than by a defect in lysosomal lipases<sup>6</sup>. Similarly, three Niemann–Pick subclasses were initially characterized as SPHINGOMYELIN-storage disorders, but it is now known that only Niemann–Pick A and B are caused by defective sphingomyelinase activity. Niemann–Pick C (NPC) is caused by the defective activity of a putative cholesterol

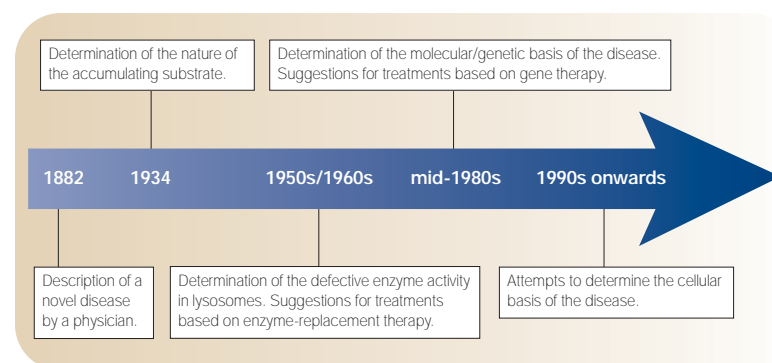
transporter, the NPC1 protein, or by defects in the soluble lysosomal cholesterol-binding NPC2 protein. Genetic evidence shows that they function together.

However, the classification of many LSDs can still be made largely on the basis of the kind of substrate that accumulates. So, in the mucopolysaccharidoses (MPS), GLYCOSAMINOGLYCANS (mucopolysaccharides) accumulate due to the impaired function of any 1 of 11 lysosomal enzymes that include exoglycosidases, sulphatases and one non-hydrolytic transferase: these lysosomal enzymes are required for the sequential degradation of glycosaminoglycans. In the sphingolipidoses, unmetabolized SPHINGOLIPIDS accumulate due to the defective activity of one of a number of enzymes (or activator proteins). And, in the oligosaccharidoses, oligosaccharides accumulate. In some cases, when more than one class of macromolecule can be a substrate because of a common determinant, a deficiency in a single enzyme can result in the accumulation of different substrates. For example, GM1 gangliosidosis and Morquio-B disease are both caused by defects in acid  $\beta$ -galactosidase activity, but they result in GM1 ganglioside and keratan sulphate accumulation, respectively, and each disease displays distinct clinical and biochemical features<sup>7</sup>. Similarly, defects in oligosaccharide degradation can result in the accumulation of glycolipids, glycoproteins or proteoglycans. For further details of these lysosomal storage disorders, see TABLE 1 and online [supplementary information S1](#) (table).

LSDs are normally monogenic (that is, they involve only a single gene), but, for most LSDs, numerous mutations have been described in the same gene in different patients. These mutations include missense, nonsense and splice-site mutations, partial deletions and insertions. Some mutations lead to the complete loss of enzyme activity, whereas others lead to reduced activity. However, no obvious genotype–phenotype correlation has been found for most LSDs and prediction of the clinical course of the disease cannot usually be made on the basis of mutational analysis. In many diseases, severe neuropathology is typical, which leads to death at an early age, whereas in other diseases, the symptoms are mainly restricted to peripheral tissues. In some diseases, individuals can be asymptomatic for the disease even though they carry a mutation that is responsible for a severe form of the disease in another individual (BOX 1). Clearly, disease severity must be related to the type of substrate that accumulates, its levels, and the tissues or cells in which it accumulates, which itself depends on whether a particular cell actually synthesizes the substrate or acquires it by active mechanisms such as phagocytosis. However, predictions about disease severity, pathology and progression, even when the mutation is documented, are almost never accurate. This reflects, to a large extent, the lack of information about the downstream secondary biochemical and cellular pathways that are altered on substrate accumulation in lysosomes. However, before discussing this issue, we briefly highlight some recent, unexpected, biochemical findings that relate to a number of LSDs.

## Box 2 | The chronology of classic lysosomal storage disorders

In the 'classic' lysosomal storage disorders (LSDs), a disease was described by a physician years before the discovery of its molecular basis (see timeline, which shows the typical history of a classic LSD combined with the dates for the relevant discoveries for Gaucher disease). Some diseases were described more-or-less concomitantly by different physicians, which is often reflected in the name of the disease (for example, Tay–Sachs disease was described in 1881 by Warren Tay and in 1886 by Bernard Sachs)<sup>98</sup>. In the case of Gaucher disease, in 1882, Phillipe Gaucher described a female patient with an enlarged spleen and also noted the presence of unusual, large cells in the spleen, which became known as 'Gaucher cells'<sup>99</sup>. During the next 50 years or so, further signs and symptoms were catalogued, but it was not until 1934 that the accumulating substance was identified as glucosylceramide. In the 1950s and 1960s, Brady and colleagues showed that glucosylceramide accumulation was caused by the defective activity of glucocerebrosidase/ $\beta$ -glucosidase<sup>100</sup>. Once the defective enzyme was discovered, the concept of enzyme-replacement therapy (ERT) was developed, in which the defective enzyme could be supplemented by active enzyme. ERT was commercialized in the early 1990s and is today used to treat more than 3,000 patients worldwide. The gene for  $\beta$ -glucosidase was localized to chromosome 1 in 1983 and was cloned in the mid-1980s and, since then, more than 150 mutations have been documented<sup>99</sup>. However, despite great progress in our understanding of the genetic, molecular and biochemical bases of this disease, virtually nothing is known about how lysosomal glucosylceramide accumulation causes disease at the cellular level.



**Classic lysosomal storage disorders.** Although it is a rather artificial classification, we define the 'classic LSDs' as those that follow the chronology of discovery that is shown in BOX 2. Therefore, initial reports, which were often as early as the late nineteenth or early part of the twentieth centuries, were limited to a description of the pathology at the tissue, and sometimes cellular, level. This was followed by determination of the nature of the accumulating substrate (usually in the middle of the twentieth century), determination of the defective enzyme and, finally, determination of the genetic and molecular bases of the disease. In some classic LSDs, new or modified substrates continue to be identified even up to the present day (see, for example, REF. 8). Some of the first LSDs to be clinically documented were the sphingolipidoses Gaucher disease and Tay–Sachs disease, which were both observed for the first time in the 1880s. Many other LSDs were subsequently described, and subclassifications of individual diseases continue to emerge (TABLE 1; see also online [supplementary information S1](#) (table)).

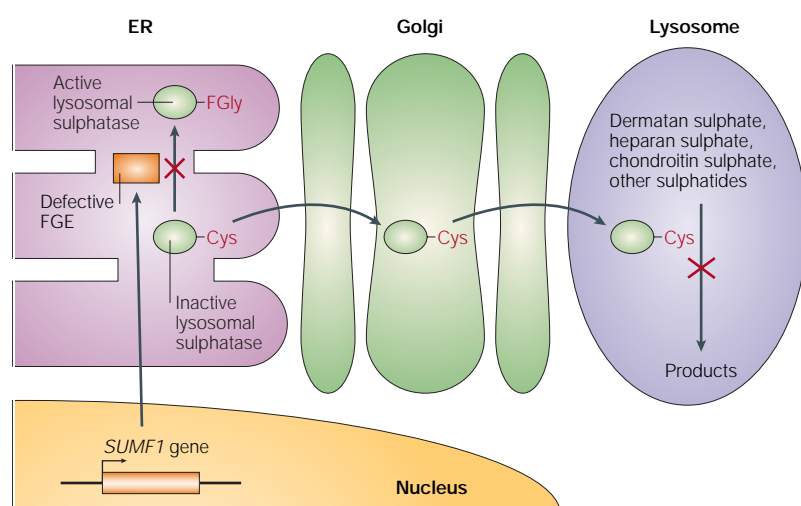
**Lysosomal storage disorders with an unexpected molecular basis.** Multiple sulphatase deficiency (MSD) was first described in the mid-1960s<sup>9</sup>. It is one of the rarest LSDs, occurring with a frequency of 1 in ~1.4 million live births.

In MSD, all of the 13 known members of the sulphatase-gene family — some lysosomal and some non-lysosomal — have a reduced activity<sup>9</sup>. Although it seemed unlikely that a disease that involves several enzymes was caused by simultaneous mutations in each of the individual enzymes, it was only recently that the molecular basis of the disease was determined. It was shown that MSD results from the failure to post-translationally convert a specific cysteine residue that is found at the catalytic centre of all sulphatases to a C $\alpha$ -formylglycine residue<sup>10</sup>. C $\alpha$ -formylglycine residues are generated in the lumen of the endoplasmic reticulum (ER) by the action of the C $\alpha$ -formylglycine-generating enzyme (FGE; FIG. 1), and mutations in the gene that encodes FGE — that is, in the *sulphatase-modifying factor-1* gene (*SUMF1*) — result in defective FGE activity<sup>11,12</sup>. At least 12 mutations in *SUMF1* have been reported<sup>11</sup>, and expression of normal *SUMF1* in MSD cells restores normal sulphatase activity and reverts the phenotype. So, as in most LSDs, MSD is monogenic, but the defective gene encodes an enzyme (FGE) that itself modifies the activity of several other enzymes (the sulphatases). One defective enzyme therefore causes a deficiency in several enzymes.

Another rare LSD, galactosialidosis, is also associated with the defective activity of more than one enzyme — that is,  $\beta$ -galactosidase and sialidase. In this disease, a multi-enzyme complex, of which the serine carboxypeptidase **cathepsin A** is the main component<sup>13</sup>, forms improperly. During its biosynthesis, cathepsin A dimerizes and associates with the precursors of sialidase and  $\beta$ -galactosidase. This association is essential to protect these glycosidases from rapid intra-lysosomal proteolysis and is also required for their correct intracellular sorting and proteolytic processing. Numerous mutations in the gene that encodes cathepsin A form the molecular basis of this LSD, which can be corrected by overexpressing cathepsin A in galactosialidosis cells<sup>14,15</sup>.

**Ceroid lipofuscinoses: many genes, one disease?** In both MSD and galactosialidosis, several enzyme defects are caused by the absence of a functional protein that is required for the stability or activity of more than one other enzyme. In the case of the neuronal ceroid lipofuscinoses (NCLs or Batten disease), defects in up to eight distinct genes lead to diseases that are characterized by a similar pathology. Symptoms include progressive neurodegeneration and the accumulation of autofluorescent material ('ceroid' and 'lipofuscin') and subunit c of ATP synthase<sup>16</sup> (except in NCL1) in the lysosomes of neurons and other cells. The NCLs comprise the most common group of progressive neurodegenerative diseases in children with an incidence as high as 1 in 12,500 live births<sup>17</sup>. Mutations have now been identified in six distinct genes/proteins<sup>16,18</sup>, and the proteins are known as CLN proteins (CLN proteins are coded by the *NCL* genes). Two (or perhaps three) of these proteins are soluble lysosomal hydrolases (CLN1, 2 and perhaps CLN5) and three or four of them are predicted transmembrane proteins (CLN3, 5, 6 and 8). Mutations in any one of these CLN proteins result in a distinct type of NCL disease. At present, little is known about CLN4 and CLN7.





**Figure 1 | The defect in multiple sulphatase deficiency.** Mutations in the *sulphatase-modifying factor-1 (SUMF1)* gene result in the production of defective  $\alpha$ -formylglycine-generating enzyme (FGE). Defective FGE cannot convert the cysteine (Cys) residue that is found at the active site of lysosomal sulphatases to  $\alpha$ -formylglycine (FGly). As a result, inactive sulphatases are transported to the lysosome, where they are unable to degrade various sulphate esters. This leads to sulphate-ester accumulation and subsequently to multiple sulphatase deficiency. Please refer to the text for further details. ER, endoplasmic reticulum.

Of the soluble hydrolases, CLN1 is protein palmitoylthioesterase-1 (PPT1)<sup>19</sup>, a hydrolase that is targeted by mannose-6-phosphate receptors to lysosomes. In lysosomes, it contributes to the degradation of palmitoylated proteins by deacylating cysteine thioesters, and the latter accumulate in NCL1 (REF 20). A mouse model that is defective in a homologue of PPT1 (PPT2) has recently been established<sup>21</sup>, but the phenotype does not correspond to the pathology of a known human LSD. This indicates that further NCL-like diseases remain to be discovered. CLN2 is tripeptidyl amino peptidase-1 (TPP1), a serine protease that cleaves tripeptides from the amino terminus of small proteins before these tripeptides are degraded by other lysosomal proteases. Less is known about the putative transmembrane proteins that cause NCL. CLN3 is an integral membrane protein of 438 amino acids with 6–11 predicted transmembrane domains, and it has recently been proposed to be an arginine transporter<sup>22</sup>. CLN6 is a unique 311-amino-acid protein with 6 or 7 transmembrane domains<sup>23,24</sup>. There is some debate about whether CLN5 is an integral membrane protein or a soluble, glycosylated protein<sup>25</sup>. At present, only chromosomal location and sequence information are available for CLN8, and the latter indicates that it is a transmembrane protein. It is not known how mutations in the eight distinct genes for the CLN proteins lead to a similar pathology and to the accumulation of similar substances in lysosomes.

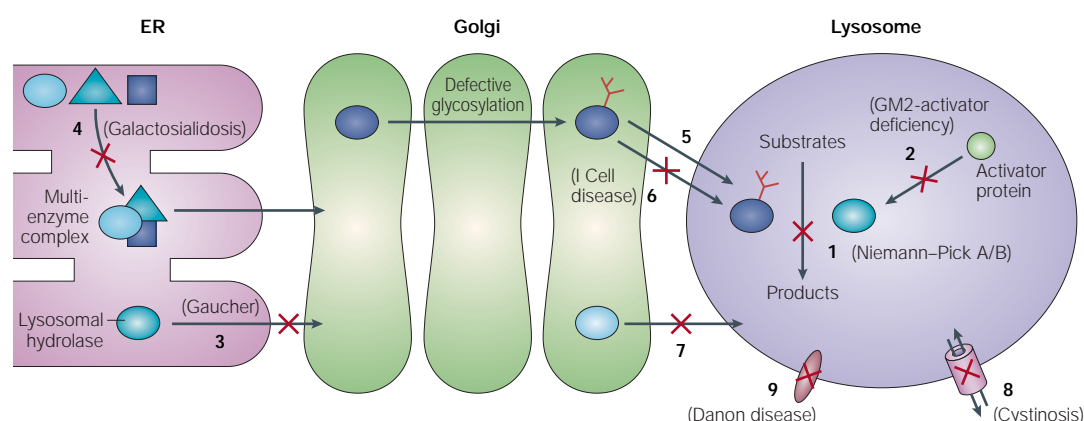
**Lysosomal storage disorders caused by defective membrane proteins.** In addition to the membrane proteins that are involved in NCL pathology, lysosomes also contain integral membrane proteins of known function, most of which are transporters that export soluble metabolites out of lysosomes. Two of these have been

implicated in LSDs so far, that is, sialin and cystinosin<sup>4,5</sup>. Sialin, which is an anion transporter, is defective in infantile sialic-acid-storage disease and Salla disease<sup>26</sup>. Cystinosin, which is a cystine transporter<sup>27</sup>, is defective in cystinosis. As several other amino acids are transported out of lysosomes, it is expected that other LSDs that are caused by defects in as-yet-unidentified amino-acid transporters will be discovered<sup>22</sup>. Similarly, defects in other lysosomal membrane proteins<sup>4</sup> presumably also lead to LSDs. Important among these might be the lysosome-associated-protein transmembrane-4 $\alpha$  (LAPTM4 $\alpha$ ), which is a putative nucleotide transporter, and the putative lysosomal ABC transporters ABC9 and ABC2 (see below). The NPC1 transporter, which is involved either directly or indirectly in cholesterol transport, has already been shown to be defective in NPC disease<sup>28</sup>. Finally, mutations in the abundant lysosomal protein lysosome-associated membrane protein-2 (LAMP2) cause Danon disease<sup>29</sup>. This disease was formerly known as 'lysosomal-glycogen-storage disease with normal acid maltase' and is characterized by the accumulation of vacuoles that contain cytoplasmic debris in skeletal and cardiac muscle. LAMP2 has one transmembrane domain, is highly glycosylated and is found ubiquitously in lysosomes. Although its precise function is unknown, it seems to have a crucial role in lysosomal stability and integrity, and in chaperone-mediated autophagy<sup>4</sup>.

**Other potential causes of lysosomal storage disorders.** In addition to the examples given above, there are numerous other defects in lysosomal proteins that can, or could, cause an LSD (TABLE 1; see also online [supplementary information S1](#) (table); FIG. 2). For example, some forms of sphingolipidoses are caused by mutations in the sphingolipid-activator proteins that are required for optimal sphingolipid hydrolysis, or in the sphingolipid-activator-protein-precursor prosaposin, although the total lack of glycolipid hydrolysis that is a result of the latter causes death and at an early age<sup>30</sup>. I Cell disease and pseudo-Hurler polydystrophy, which were originally classified as mucopolysaccharidoses, are now known to be caused by the defective transport of lysosomal enzymes due to defects in the gene that encodes the UDP-*N*-acetylglucosamine:lysosomal enzyme *N*-acetyl-glucosaminyl-1-phosphotransferase. This enzyme catalyses the first step in the formation in the Golgi apparatus of mannose-6-phosphate (REF 6) — a key recognition marker for targeting many hydrolases to lysosomes. In these diseases, lysosomal enzymes are secreted, rather than being targeted to the Golgi apparatus, and the fact that not all cells are deficient in their lysosomal enzyme content in these diseases led to the proposal that there are mannose-6-phosphate-independent pathways of lysosomal sorting<sup>6,31</sup>. So, studying the cellular abnormalities in LSDs can lead to advances in our understanding of normal cellular processes. Finally, even though none has been described so far, it is plausible that defects in other components of transport pathways to and from lysosomes, either in adaptor proteins, coat proteins or even lysosome-specific SNAREs (soluble NSF (*N*-ethylmaleimide-sensitive fusion protein) accessory protein (SNAP) receptors),

#### ABC TRANSPORTERS

A family of membrane transport proteins that use the energy of ATP hydrolysis to transport various molecules across the membrane.



**Figure 2 | The biochemical and cellular basis of lysosomal storage disorders.** Most mutations in 'classic' lysosomal storage disorders (LSDs) result in the delivery of a defective enzyme that has a reduced catalytic activity to lysosomes (label 1). In some cases, another protein that is required for optimal hydrolase activity is defective or absent (label 2). An LSD can be caused by the defective transport of a lysosomal hydrolase out of the endoplasmic reticulum (ER) due to a mutation that causes misfolding (label 3). Alternatively, an LSD can be caused by the defective transport of a lysosomal hydrolase out of the ER because a multi-enzyme complex that is required for transport cannot form (label 4). In the Golgi, defective glycosylation could result in an enzyme with reduced catalytic activity (label 5). Alternatively, defective glycosylation in the Golgi could produce an enzyme that cannot reach lysosomes because it cannot bind to mannose-6-phosphate receptors (due to defective glycosylation with mannose-6-phosphate; label 6). Defects in other transport steps from the Golgi could also lead to an LSD (label 7). Several LSDs are caused by defects in integral lysosomal membrane proteins. These include defects in transporters (label 8), or in proteins that are involved in other vital regulatory events of lysosomal function (label 9). In this figure lysosomal hydrolases are shown in various shades of blue, and a relevant LSD example is shown for each defect when one is known. For further details, please refer to the main text.

could mis-target lysosomal hydrolases or membrane proteins and therefore cause an LSD (FIG. 2). Whether mutations in the proteins that are involved in such pathways would result in a viable phenotype remains to be established.

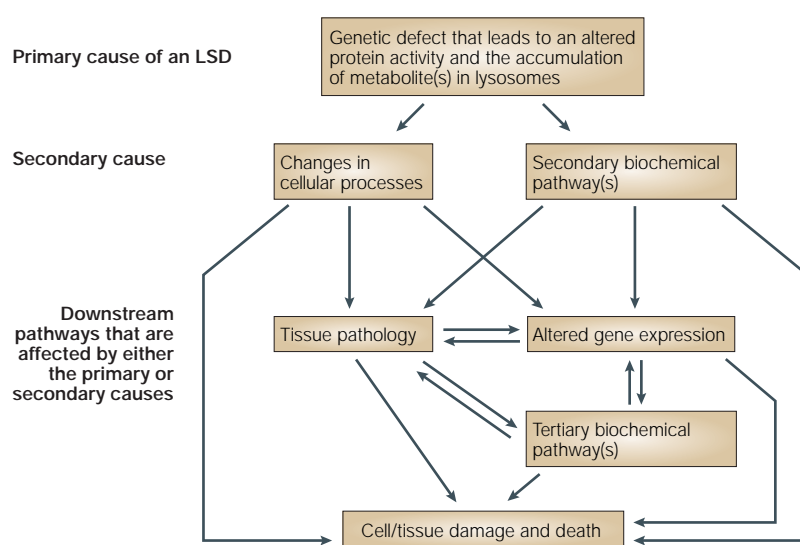
The cell biology of lysosomal storage disorders. The main open question in the biology of LSDs concerns delineation of the biochemical and cellular pathways that cause disease pathology (FIG. 3). LSDs are all characterized by the intra-lysosomal accumulation of unmetabolized substrates, which is the primary cause of disease, but the extensive range of disease symptoms indicates that many secondary biochemical and cellular pathways must also be activated. As no two diseases share exactly the same pathology, different pathways are presumably activated in different diseases. This latter point is not entirely obvious as cells could, in principle, have an 'intra-lysosomal protein response' that is analogous to the 'UNFOLDED PROTEIN RESPONSE' in the ER, in which similar pathways are activated irrespective of the nature of the unfolded protein<sup>32</sup>. Indeed, such a response might occur in LSDs, but specific responses that are peculiar to each LSD or to the different metabolites probably ultimately determine the pathology of the specific LSDs. So, the primary accumulating metabolite affects a secondary biochemical or cellular pathway, which then subsequently causes tissue pathology, altered gene expression, and the activation of tertiary biochemical pathways (FIG. 3). Any of these events could be the main cause of tissue damage and death in LSDs, but surprisingly little is known about the identity of these pathways or their regulation in LSDs.

**Lysosome stability.** One straightforward explanation for LSD pathology would be that lysosomal stability, integrity or permeability is compromised, which would lead to the release of hydrolases and the accumulating metabolites into the cytosol. Unfortunately, there is very little evidence to support this idea. Indeed, ever since intracellular storage material was visualized in LSDs, innumerable studies have reported the accumulation of storage material within membrane-bound compartments, which are presumably lysosomes. However, the possibility cannot be excluded that some lysosomal components, such as storage materials and hydrolases, leak out into the surrounding cytoplasm. And, although this would not represent the bulk of the accumulating material, it might be sufficient to account for at least part of the pathology. There is evidence that lysosomal proteins can leak into the cytosol under various physiological and pharmacological conditions. For example, lysosomal proteases such as cathepsins are found in the cytosol during apoptosis<sup>33</sup>, LYSOSOMOTROPIC AGENTS induce apoptosis by inducing lysosomal leakage<sup>34</sup>, and the expression of certain proteins (such as amyloid A $\beta$ 1–42) results in the appearance of lysosomal enzymes (such as cathepsin D and  $\beta$ -hexosaminidase) in the cytosol<sup>35</sup>.

Lysosomes also contain a number of integral membrane proteins of unknown function<sup>4</sup>, and some of these could function to pump metabolites out of lysosomes on their accumulation in LSDs. Of particular interest in this regard are the putative lysosomal ABC transporters ABC9 (REF. 36) and ABC2 (REF. 37). ABC-protein-family members have been proposed to have roles in lipid translocation<sup>38</sup>, and ABC2 has been proposed to be a regulator of neuronal-transmembrane-lipid transport,

**UNFOLDED PROTEIN RESPONSE**  
A cellular response that is triggered by the accumulation of misfolded proteins in the endoplasmic reticulum (ER) and that results in the transcriptional upregulation of ER chaperones and degradative enzymes.

**LYSOSOMOTROPIC AGENTS**  
Molecules that move to the lysosome: mostly weak bases that diffuse across the lysosomal membrane as uncharged molecules and are trapped inside in their protonated form due to the low pH.



**Figure 3 | A possible roadmap of the pathology of lysosomal storage disorders.** Lysosomal storage disorders (LSDs) are all characterized by the intra-lysosomal accumulation of unmetabolized substrates, which is the primary cause of the disease. However, the extensive range of disease symptoms indicates that many secondary biochemical and cellular pathways must also be activated. So, the primary accumulating metabolite affects a secondary biochemical or cellular pathway, which then subsequently causes tissue pathology, altered gene expression and the activation of tertiary biochemical pathways. Please refer to the main text for further details.

specifically cholesterol and galactosylceramide transport<sup>39</sup>. However, no direct data support this idea. Furthermore, it is hard to see how a lysosomal ABC transporter would be involved in pumping material out of lysosomes, because all mammalian ABC transporters transport their substrates from the cytosol to the lumen of an organelle or to the extracellular environment. Interestingly, impaired lipid efflux through a non-lysosomal ABC transporter, **ABC1**, has been proposed to be involved in the pathological mechanism of NPC disease<sup>40</sup>.

In addition, lysosomes can fuse with the plasma membrane in a  $\text{Ca}^{2+}$ -regulated fashion<sup>41–43</sup>, which results in the secretion of several proteins into the extracellular space, for example, cathepsin B<sup>44</sup>. Could the fusion of lysosomes also occur with intracellular organelles other than just late endosomes? Again, there is no direct evidence for this, but the close juxtaposition of lysosomes with a number of intracellular organelles might indicate that such events are possible. If this does occur, then it could have disastrous consequences for the organelles with which lysosomes fuse.

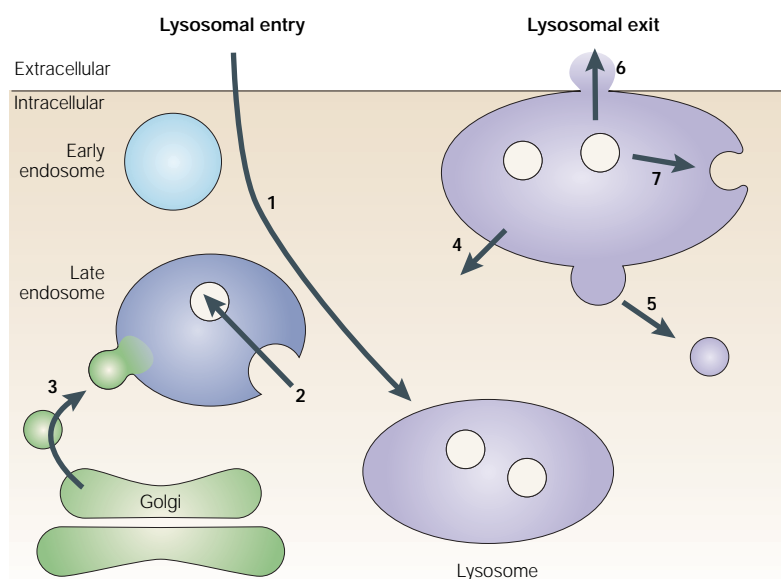
**Defective intracellular trafficking.** Another relatively straightforward explanation for LSD pathology would be that the accumulation of undegraded metabolites in lysosomes blocks intracellular transport to or from lysosomes due to lysosomal storage capacity ‘overload’ (BOX 3). Although it is difficult to envisage such feedback and feed-forward pathways for soluble metabolites that accumulate in the lysosomal lumen, it is easier to imagine them for membrane lipids, such as those that accumulate in the sphingolipidoses. Indeed, a common defect in lipid sorting and transport among the different sphingolipidoses has been proposed<sup>45,46</sup>, and this was

based on the observation that a short-acyl chain (Bodipy) derivative of lactosylceramide is targeted to the Golgi apparatus in normal cells, but to endosomes and lysosomes in cells from sphingolipidosis patients<sup>47,48</sup>. This process is linked to the accumulation of unesterified cholesterol<sup>49</sup> and to the activity of the small GTPases RAB7 and RAB9 (REF. 50) in these cells. The fact that cholesterol and sphingolipid levels are inter-related in LSDs is hardly surprising because both are important components of microdomains/rafts at the cell surface<sup>51,52</sup>. So, it is clear that the accumulation of one will have profound effects on the other. Indeed, changes in cholesterol levels are observed in LSDs that are caused by defective sphingolipid hydrolysis, such as Niemann–Pick A and B. Furthermore, changes in sphingolipid sorting and transport are observed in NPC, an LSD that is caused by defective cholesterol transport and in which levels of cholesterol accumulation are closely connected to sphingolipid (ganglioside) accumulation<sup>53</sup>. Whether the primary pathology in NPC is caused by accumulating sphingolipids or by cholesterol is unknown at present. However, both sphingolipids and cholesterol have crucial roles in neuronal function<sup>54,55</sup>, which is where the pathology of NPC and most sphingolipidoses is manifested.

Little is known about defective protein sorting in LSDs, with the exception of those diseases that are directly caused by a defect in protein sorting, such as I Cell disease and pseudo-Hurler polydystrophy<sup>6</sup>. Recently, a defect in the sorting of the GM2-activator protein and of prosaposin, the precursor of the other sphingolipid-activator proteins, to lysosomes was detected in cell lines that contained a dominant-negative construct of sortilin<sup>56</sup> (a transmembrane Golgi protein that is required for the sorting and transport of some proteins to lysosomes). However, no human LSD has been associated with this pathway so far. Some interesting studies have also recently documented the effects of expressing or mutating proteins that cause LSDs in *Saccharomyces cerevisiae* and *Caenorhabditis elegans*, model systems that should shed light on the physiological and pathological functions of these proteins. Ablation of the gene that encodes **Btn1** — a yeast orthologue of the human CLN3 protein that is responsible for an NCL (TABLE 1; see also online [supplementary information S1](#) (table)) — causes changes in the vacuolar pH in yeast<sup>57</sup> (the yeast vacuole corresponds to the mammalian lysosome). And, a protein that is upregulated in Btn1-deficient yeast (**Btn2**) interacts with **Yif1**, which is a component of a protein complex at the Golgi that functions in ER-to-Golgi transport<sup>58</sup>. In *C. elegans*, mutations in CUP-5 — a homologue of mucolipin-1, which is the protein involved in ML IV pathology — result in enhanced rates of fluid-phase-marker uptake, decreased degradation of endocytosed proteins, accumulation of large vacuoles<sup>59</sup> and an excess of apoptotic cells<sup>60</sup>. Mucolipin-1 has also recently been suggested to be a multiple-subconductance, non-selective cation channel that might have a role in normal endosomal function<sup>61</sup>. Together, these findings indicate that unexpected discoveries regarding abnormal intracellular trafficking in LSDs might soon emerge.



## Box 3 | Lysosomes and lysosomal storage disorders



Material that is to be degraded is exposed to hydrolytic enzymes in the lysosomal lumen, and the products return to the cytosol to function as new building blocks. Cells use intricate molecular machines to present the substrates to the hydrolytic enzymes and to expel the residual material. In lysosomal storage disorders (LSDs), molecules accumulate that cannot be expelled. The molecular machines that are responsible for lysosomal uptake and release are therefore potential targets for LSD therapy.

Extracellular material and membrane components enter the lysosome through endocytotic vesicles and endosomes (see figure, label 1). Late endosomes presumably fuse with lysosomes<sup>101</sup>. Various multi-component protein complexes<sup>102</sup> sort membrane (glyco)proteins and lipids into membrane invaginations that bud into internal 50–80-nm diameter vesicles, which are the actual platforms for hydrolysis<sup>2,103</sup> (label 2). Cytoplasmic material enters the lysosome as the content of the invaginating vesicles or by being engulfed into autophagic vacuoles that subsequently fuse with the lysosome. The vacuolar-type H<sup>+</sup>-ATPase, hydrolytic enzymes and co-factors are sorted at the Golgi into vesicles that are targeted to the late endosomes (label 3). One sorting pathway involves the binding of soluble lysosomal proteins by mannose-6-phosphate receptors. On the plasma membrane, these receptors retrieve missorted or exogenously added (by enzyme-replacement therapy) lysosomal enzymes from the extracellular environment.

Clearly, there are pathways out of lysosomes. Lysosomes contain transporters that translocate sugars, amino acids and lipids across the limiting membrane (label 4). 'Retrograde' transport vesicles return the relevant SNAREs (soluble NSF (*N*-ethylmaleimide-sensitive fusion protein) accessory protein (SNAP) receptors) and other transport proteins to earlier compartments<sup>101</sup> (label 5). Lysosomes (and multivesicular bodies) can fuse with the plasma membrane<sup>41,104</sup> (label 6). Many cells have developed secretory lysosomes<sup>105</sup>, which release their internal vesicles as 'EXOSOMES'. Internal vesicles might fuse back with the limiting membrane<sup>106</sup> (label 7). Agents like hydrophobic amines block the routes out of the lysosome and induce membrane storage. Drugs that promote outward transport might alleviate the clinical development of some LSDs.

**Defective intracellular signalling.** In at least two of the sphingolipidoses, defective signalling as a result of changes in the production of ceramide — an important lipid second messenger<sup>62,63</sup> — could have a role in the development of pathology. In Niemann–Pick A and B diseases, which are caused by the defective activity of **acid sphingomyelinase**, ceramide is not produced in response to various ligands. As ceramide that is produced by acid sphingomyelinase is thought to be a key second messenger in apoptosis, a lack of ceramide production would

have profound effects on cell physiology. For example, in acid-sphingomyelinase-knockout models, changes in apoptosis at the cell and tissue level have been observed<sup>64,65</sup>. Farber disease, which is caused by a deficiency in ceramidase activity, results in ceramide accumulation<sup>66</sup>. In these diseases, it has, so far, been difficult to correlate changes in ceramide levels directly with disease progression. As other sphingolipids (for example, lysosphingolipids) are also thought to be first or second messengers, defective intracellular signalling might eventually be recognized as being crucial to our understanding of LSD pathology — at least in the sphingolipidoses and other LSDs in which sphingolipids accumulate as secondary metabolites.

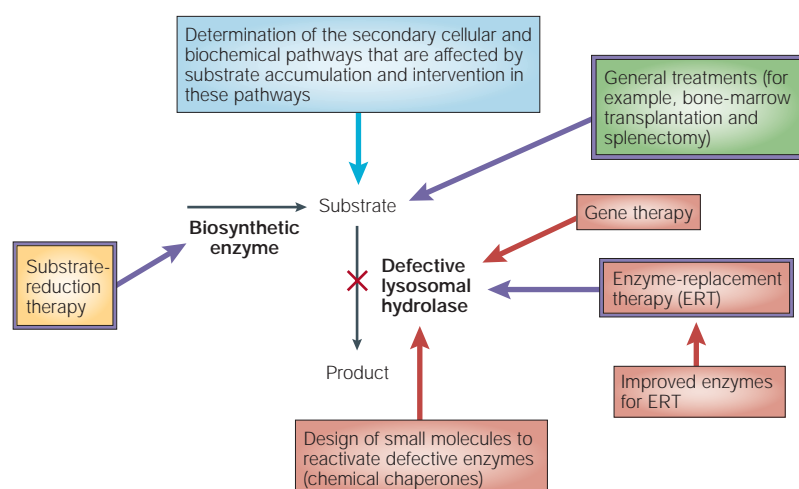
**Secondary biochemical pathways.** The identification of secondary metabolites in the LSDs has often been the result of attempts to identify serum markers that would be of use in following disease progression and in intervention — in particular, for those diseases that are mainly characterized by peripheral symptoms. For example, changes in the levels of serum chitotriosidase<sup>67</sup> or chemokine CCL18 (REF 68) are characteristic of Gaucher disease, but the relationship between glucosylceramide accumulation and the secretion of these compounds is unknown. Attempts have also been made to determine changes in secondary metabolites in the brain. For example,  $\alpha$ - and  $\beta$ -synucleins accumulate in the brains of mouse models of the GM2 gangliosidosis<sup>69</sup>, and the levels of phospholipids change in several LSDs in a tissue-specific manner<sup>70</sup>. Recently, the rate-limiting enzyme in the pathway of phosphatidylcholine synthesis (**CTP:phosphocholine cytidylyltransferase**) has been shown to be directly activated by glucosylceramide<sup>71</sup> in a model of neuronopathic Gaucher disease. In this model, the accumulation of glucosylceramide correlated with increased rates of phosphatidylcholine synthesis. Finally, some studies have indicated that defective Ca<sup>2+</sup> homeostasis might be responsible for the neuropathophysiology of some LSDs, such as Gaucher disease<sup>72</sup>, Sandhoff disease<sup>73</sup> and ML IV (REF 74). The crucial roles<sup>75</sup> of calcium in normal brain physiology make this an attractive proposition.

**Altered gene expression.** Gene profiling, mainly using gene microarrays, has provided the first information on the changes that occur in gene expression in several LSDs. In the spinal cord of Sandhoff mice, genes that are related to an inflammatory process that is dominated by activated microglia are upregulated<sup>76</sup>. Similar changes have been observed in the cerebral cortex from a Tay–Sachs patient and a Sandhoff patient by serial analysis of gene expression<sup>77</sup>. These changes are supported by a biochemical analysis that showed increased levels of a number of inflammatory markers in the brain of mouse models of GM1 and GM2 gangliosidosis<sup>78</sup>. Together with morphological changes, these findings led to a proposal for the pathological mechanism of these GM2 gangliosidosis, which involves neuronal cell death due to ganglioside storage, followed by microglial activation and, as a consequence, reactive gliosis<sup>77</sup>. Similar conclusions were reached for the unrelated LSDs MPS I

## EXOSOMES

50–80-nm membrane vesicles that are secreted into the extracellular milieu as a consequence of multivesicular-body fusion with the plasma membrane.





**Figure 4 | Therapies for lysosomal storage disorders.** Lysosomal storage disorder (LSD) treatments can be divided into those that directly modify the defective enzyme (red), those that reduce the levels of biosynthesis of the accumulating substrate (yellow), general treatments that deal mainly with the symptoms (green) and potential new therapies that will be based on intervening in downstream cellular pathways that are modified in LSDs (blue). Treatments that are framed in purple are in clinical use at present. With respect to treatments that directly modify the defective lysosomal hydrolase, recent studies have focused on the design of small molecules that are able to reactivate defective enzymes<sup>90,91</sup>. The study of these so-called ‘chemical chaperones’ has received impetus from work showing that defective  $\beta$ -glucosidase could be reactivated in a Gaucher-disease model<sup>107</sup>. However, a significant amount of work is still required before this approach can become a real therapeutic option for LSDs.

and MPS IIIB, in which gene profiling of the cerebral cortex of mouse models also indicated the involvement of activated microglia<sup>79</sup>.

Although these studies led to proposals of pathophysiological mechanisms, gene profiling is not always definitive. For example, in the *CLN3*-knockout mouse, which is a mouse model of NCL3, changes in the expression of 756 gene products in the cerebellum of 10-week-old mice were detected. These were divided into 14 functional categories, including genes involved in neurotransmission, neuronal cell structure and development, immune response and inflammation, and lipid metabolism<sup>80</sup>. Mitochondria were previously thought to be involved in the pathology of NCL<sup>16</sup>, but surprisingly few changes were detected in mitochondrial gene expression. By contrast, significant changes were observed in the expression of genes that are involved in lipid metabolism, which is consistent with the accumulation of lipopigments in the NCLs. A cursory analysis of other genes that were altered in the *CLN3*-knockout mouse highlights changes in the levels of proteins that are involved in lipid signalling (such as sphingosine kinase) and in intracellular trafficking (such as the coatamer  $\gamma$ -subunit ( $\gamma$ -COP) and ER-Golgi intermediate compartment 53kDa protein (ERGIC53)). However, the number of gene-expression changes is overwhelming and almost precludes intelligent analysis, and, by itself, cannot provide mechanistic information about pathology. Indeed, gene profiling after disease development does not provide information about the temporal sequence of gene up- or downregulation, and therefore cannot provide information about the primary cause of

disease pathology. A comprehensive database is required that documents the changes in gene expression that occur at the different stages of disease progression, in different tissues, and in different diseases that show similar or divergent pathologies. An example of this kind of approach is the comparison of the gene-expression changes in MPS I and MPS IIIB mouse models. Thirteen common genes were upregulated in both mouse models<sup>79</sup>, but whether these genes are responsible for the pathology that is shared by these two LSDs is unknown at present. However, this kind of comparative approach is an important direction to pursue in the future.

**Therapies for lysosomal storage disorders**  
Treatments for LSDs can be broadly divided into those that address the symptoms and those that address the cause (FIG. 4). Of the treatments for symptoms, splenectomy (surgical removal of the spleen) used to be relatively common, at least for Gaucher-disease patients. Splenectomy can reduce the symptoms, such as thrombocytopenia (low platelet count) and anemia, that accompany some LSDs, but does not cure the disease. Bone-marrow transplantation is also sometimes carried out. Part of the logic for this is based on the finding that a small, but significant, fraction of lysosomal enzymes are secreted by normal cells. So, transplanted bone marrow will produce cells that secrete normal enzymes, and these normal enzymes will subsequently be internalized by affected cells. Other specific treatments are available for particular diseases. For example, chronic haemodialysis and/or renal transplantation are used for Fabry disease because renal insufficiency is a common complication. However, it should be stressed that for most of the rarer LSDs, no specific or definitive treatment is available at present, and the medical options are limited to disease management rather than therapy. Without a detailed understanding of the precise mechanisms that operate in each LSD, and without easy and early means of diagnosis<sup>81</sup>, new and successful treatments will not become available.

In addition to these general treatments for the symptoms of LSDs, there are several potential therapeutic options that deal directly with the cause of the disease, either by replacing the defective gene or enzyme or by directly targeting the systems that are affected by the accumulation of undegraded metabolites. These options are discussed in a number of online resources, which also give further important information about LSDs (BOX 4).

**Gene therapy.** The most effective treatment for LSDs, and for any other inherited metabolic disease, is somatic gene therapy. However, the likelihood of gene therapy becoming a viable option in the near future — and perhaps even in the distant future — remains small. Nevertheless, as LSDs are monogenic, and as relatively small levels of lysosomal enzymes are expected to be sufficient to correct these diseases, significant resources are being channelled into research on gene therapy for the LSDs. Briefly, several *ex vivo* and *in vivo* gene-transfer methods have been used to transfer the relevant genetic material into defective cultured cells efficiently and, as a consequence, the enzyme

## Box 4 | Useful online resources for lysosomal storage disorders

Detailed information about lysosomal storage disorders (LSDs) and disease therapy can be found at numerous online resources:

- The Mendelian Inheritance in Man web site is a catalogue of human genes and genetic disorders, and it includes information on LSDs (<http://www3.ncbi.nlm.nih.gov/Omim>).
  - LSDs have also been specifically grouped together at the Inborn Errors in Metabolism web site (<http://www.med.unibs.it/%7Emarchesi/inborn.html>).
  - Human gene mutations are listed in the Human Gene Mutation Database, which collates information on published gene lesions that are responsible for human inherited disease (<http://www.hgmd.org/>).
  - The European Study Group on Lysosomal Storage Diseases (ESGLD) maintains a web site (<http://www.esgld.org/>).
  - The Global Organisation for Lysosomal Diseases is a non-profit international collaboration for individuals and carers affected by LSDs, and it has a web site that gives relevant information on LSDs and further useful links (<http://www.interactivhealth.com/gold>).
- In addition, many individual diseases have their own web sites, some of which are patient oriented and some of which are more scientifically oriented. For example:
- Gaucher disease (<http://www.gaucher.org.uk> and <http://www.childrensgaucher.org>).
  - Tay–Sachs disease (<http://www.ntsad.org>).
  - Mucopolysaccharidoses ([http://www.ninds.nih.gov/health\\_and\\_medical/disorders/mucopolysaccharidoses.htm](http://www.ninds.nih.gov/health_and_medical/disorders/mucopolysaccharidoses.htm)).
  - Batten disease (<http://www.bdsra.org> and <http://www.ucl.ac.uk/ncl>).

activity has been reconstituted. However, little progress has been made in applying these methods to humans or to animal models. This is true for almost every LSD for which the identity of the defective gene is known. As this area has been reviewed extensively elsewhere (see, for example, REFS 82,83), we will not address it further here.

**Enzyme-replacement therapy.** The most successful treatment that is available for the LSDs at present is enzyme-replacement therapy (ERT). The main problem to be overcome in this treatment is how to target the enzyme to the defective cells. In Gaucher disease, for which ERT has been available since 1990, recombinant  $\beta$ -glucosidase can be targeted to macrophages by remodelling its oligosaccharide chains to expose core mannose residues. This modified enzyme is endocytosed after it binds to cell-surface mannose receptors on macrophages and is subsequently delivered to lysosomes where it supplements the defective enzyme<sup>84</sup>. The importance of uptake by mannose receptors is reinforced by studies showing that upregulation of the mannose receptor can improve the delivery of recombinant  $\beta$ -glucosidase to Gaucher macrophages<sup>85</sup>, and can therefore improve the efficacy of ERT. In other LSDs, different cell-surface receptors, such as mannose-6-phosphate receptors<sup>86</sup>, might be required for the successful uptake of lysosomal enzymes. At present, ERT has been approved for Fabry disease<sup>87,88</sup> and MPS I. ERT for other diseases — such as MPS II, MPS VI and MPS VII — is progressing and it might one day be available for all LSDs<sup>89</sup>, at least for those in which the symptoms are mainly restricted to the peripheral systems (as the blood–brain barrier is impermeable to the enzymes used in ERT).

Attempts are being made to improve the efficacy of ERT<sup>90,91</sup>. This might include better targeting to the affected cells, and the improved stability or catalytic efficiency of recombinant enzymes. For the latter, knowledge of the three-dimensional structure of the affected enzyme

is crucial, and the structures of  $\beta$ -glucosidase<sup>92</sup> and  $\beta$ -hexosaminidase B<sup>93</sup> have recently been determined.

**Specific drug therapy.** Other treatments for LSDs are based on preventing the metabolic and cellular defects that occur on the accumulation of undegraded substrates, or on preventing the accumulation of undegraded substrates by partially inhibiting synthesis (FIG. 4). The latter has been attempted for the sphingolipidoses, in which inhibition of glycolipid synthesis by *N*-butyldeoxynojirimycin blocks the development of disease symptoms in Tay–Sachs-disease mouse models<sup>94</sup> and in Gaucher-disease patients<sup>95,96</sup>. (*N*-butyldeoxynojirimycin is an inhibitor of glucosylceramide synthase, which is the first enzyme in the pathway of GLYCOSPHINGOLIPID synthesis.) Presumably this treatment, which is known as ‘substrate deprivation’ or ‘substrate-reduction therapy’ (SRT), will be effective for any of the sphingolipidoses in which glycolipids accumulate, in particular, for those in which pathology develops in the brain (these small molecules, in contrast to the enzymes that are used in ERT, do cross the blood–brain barrier). However, the lack of long-term studies on the effects of depleting glycolipid levels — in particular, during development, when these lipids have crucial roles — might prohibit applying this approach at least *in utero* or to children. But, it is the first new treatment for Gaucher disease in more than a decade<sup>91</sup>, and issues that relate to the ease of use of SRT for patients might eventually render this a real option for the treatment of sphingolipidoses and, in principle, for the treatment of other LSDs in which inhibition of synthesis would decrease the levels of accumulating undegraded substrates.

There is also a relatively successful therapy for cystinosis that uses cysteamine, which causes the depletion of lysosomal cystine levels<sup>97</sup>. The mechanism by which cysteamine mediates its effects is complex, but it involves the uptake of cysteamine into lysosomes through a specific

## GLYCOSPHINGOLIPID

A sphingolipid with an oligosaccharide headgroup. The sugar that is linked to the ceramide lipid backbone is generally glucose, but is galactose in galactosylceramide and sulphatide.

transporter, its reaction with cystine to form the mixed disulphide cysteamine–cysteine, exit of this compound from lysosomes through a transporter, and its reduction to cysteamine and cysteine by glutathione in the cytoplasm. This therapy has made cystinosis one of the most treatable LSDs<sup>97</sup>.

Finally, new drug therapies should emerge as the downstream biochemical and cellular pathways that are affected in LSDs are delineated. Unfortunately, and as has been emphasized throughout this article, very little is known in this regard for most LSDs. However, we anticipate that new treatments, which could be used alone or in combination with existing treatments, will become available when cell biologists face the challenge of understanding the cellular basis of this fascinating class of inherited metabolic disorders.

## Conclusions

In this review, we hope that we have convinced readers that the time is ripe for a concerted effort to determine the cellular basis of the pathology of LSDs. In particular, this is highlighted by the recent identification of the primary cause of some LSDs (such as MSD and some NCLs), and the availability of a large number of cell lines that have been derived from different tissues, animal models and even human patients that are defective in the activity of one or more lysosomal proteins. Furthermore, because so little is known about how the intra-lysosomal accumulation of undegraded metabolites leads to cell and tissue dysfunction, the study of LSDs will almost certainly lead to the discovery of new and unexpected pathways that are involved in the regulation of normal cell physiology.

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Competing interests statement  
The authors declare that they have no competing financial interests.

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