Lysosomal Storage Diseases

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Competing interests

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Abstract

Lysosomal storage diseases (LSDs) are a group of over 70 diseases characterized by lysosomal dysfunction, most of which are inherited as autosomal recessive traits. These disorders are individually rare but collectively affect 1 in 5,000 live births. LSDs typically present in infancy and childhood, although adult onset forms also occur. Most LSDs have a progressive neurodegenerative clinical course, although symptoms in other organ systems are frequent. The mutated genes in LSDs encode proteins that have roles in different lysosomal functions, including lysosomal enzymes and lysosomal membrane proteins. The lysosome is the key cellular hub for macromolecule catabolism, recycling and signalling and defects that impair any of these functions cause the accumulation of undigested or partially digested macromolecules in lysosomes (that is, 'storage') or impair the transport of molecules, which can also result in cellular damage. Consequently, the cellular pathogenesis of these diseases is complex and currently incompletely understood. Several LSDs are now treatable with approved, disease-specific therapies, mostly based on enzyme replacement. However, small molecule therapies, including substrate reduction and chaperone therapies, have also been developed and approved for some LSDs, whereas gene therapy and genome editing are at advanced preclinical stages and, for a few disorders, have already progressed to the clinic.

[H1] Introduction

Lysosomal storage diseases (LSDs) are heritable (inborn) errors of metabolism that affect the function of the lysosome. LSDs comprise a group of 70 monogenic disorders of lysosomal catabolism, most of which are inherited as autosomal recessive traits, but three are X-linked. These disorders are caused by mutations in genes encoding lysosomal proteins, such as lysosomal glycosidases, proteases, integral membrane proteins, transporters, enzyme modifiers or activators. Genetic mutations in lysosomal genes affects the function of the encoded protein product, resulting in lysosomal malfunction and the gradual accumulation of substrates inside the lysosome (that is, 'storage'), which, ultimately leads to cell dysfunction and cell death. Of the ~1,300 genes involved in lysosomal function, many monogenic disorders have been described, including 50 enzyme deficiencies (which can be subclassified according to the biochemical type of stored material for example, the sphingolipidoses, mucopolysaccharidoses and glycoproteinoses, 7 disorders involving integral

membrane proteins, 12 disorders of lysosome-related organelles, and 14 disorders producing ceroid lipofuscinosis (TABLE 1) [1,2,3,4,5,6].

LSDs are genetically and clinically heterogeneous disorders (FIG. 1 and TABLE 1) but frequently present as paediatric neurodegenerative diseases that are often accompanied by visceromegaly (the enlargement of abdominal organs such as liver and spleen)[7]. However, depending on the specific genetic defect and the biochemical nature of the macromolecules stored, LSDs can also cause skeletal dysmorphia due to bone pathology, and can be associated with developmental delay or various other central nervous system (CNS) deficits, in addition to symptoms affecting other organ systems (FIG. 1 and TABLE 1). Patients present with a continuum of disease severity that loosely correlates with the type of mutation and residual activity of the mutant protein, but they are generally classified based on the type of disorder, and the age of onset of the clinical signs as congenital or infantile (which usually have the most severe presentation), late infantile, juvenile and adult types. Diagnosis of LSDs is based on clinical symptoms and confirmation of increased storage or genetic alterations using several diagnostic tests, including enzymatic analysis of body fluids and single gene sequencing[7]. However, diagnosis, especially of milder cases with longer survival, is often delayed due to clinical symptoms that are characteristic of other, more common, conditions. More recently, next-generation sequencing, especially whole exome sequencing, is becoming routine and might reduce the time from presentation to diagnosis.

Our understanding of the pathophysiology of LSDs has undergone major advances that have identified multiple potential clinical intervention points. Through the use of animal models, various therapies have been evaluated *in vivo* and have progressed to clinical trials and regulatory approval. Enzyme replacement therapy (ERT) to restore defective enzymes is the cornerstone of current treatment paradigms for some LSDs, but small molecule therapies that reduce storage by inhibiting the production of the substrates that are stored are also approved, and are an expanding area of drug development. Nucleic acid-based medicines (such as gene replacement, antisense oligonucleotide therapies, or gene editing) are also emerging. As more treatments for LSDs become available, adding these conditions to newborn screening panels should speed the diagnosis, improve the clinical care of patients and aid in the prevention of LSDs in subsequent pregnancies. This Primer provides an overview of the LSDs, the epidemiology and genetics of these disorders, in addition to current insights into their pathophysiology and the present status of therapies.

[H1] Epidemiology

[H2] Incidence and prevalence.

As a group, LSDs are common with an estimated incidence of 1 in 5,000 to 1 in 5,500 for LSDs that involve lysosomal enzyme or integral membrane protein defects; however, individual LSDs are rare, with estimated incidences ranging from 1 in 50,000 to 1 in 250,000 live births[8]. The most common LSDs are Fabry disease (up to 2.5 cases per 100,000 males), Gaucher disease (up to 2.0 cases per 100,000 individuals), metachromatic leukodystrophy (up to 2.5 cases per 100,000 individuals) and Pompe disease (up to 2.5 cases per 100,000 individuals) (reference not clear how this can be added from endnote so assume Louise you number this on final draft and insert link in ref list?). In addition the neuronal ceroid lipofuscinoses (NCLs) are relatively common, with an incidence of 1 per 100,000 individuals in the general population, with a higher incidence in Finland owing to a founder effect (CLN3, CLN5 and CLN8)[9,10]. These values are undoubtedly underestimates, as calculations of incidence presume that all cases are ascertained or that accurate carrier frequencies are known. Although these factors are known for the more severe subtypes of LSDs with infantile presentations, such as Tay-Sachs disease in the Ashkenazi Jewish population, it might not be the case for pan-ethnic or juvenile-onset and adult-onset LSDs, whereby many individuals are diagnosed up to decades after symptom onset, and some individuals never receive a diagnosis. Despite a low incidence of individual LSDs in general, the incidence can be higher in specific ethnicities, often based on a founder effect (TABLE 2). For example, aspartylglucosaminuria (one of the glycoproteinoses) is a rare condition worldwide (the exact incidence is estimated as <0.2 cases per 100,000 individuals) that is common in the Finnish population (estimated carrier frequency of 2.3 to 3%) owing to a founder effect and genetic isolation[11]. Similarly, Hermansky-Pudlak syndrome (a lysosome-related organelle (LRO) disorder) is found in 1 in 500,000 individuals worldwide, but in ~1 in 1,800 residents of northwestern Puerto Rico[12,13].

[H2] Effect of survival and treatment.

The prevalence of a LSD is determined by the incidence of the disorder, which varies in different populations, and the average survival for that disorder. The advent of approved therapies for some LSDs and/or more effective supportive care for patients, most notably the use of feeding tubes and better pulmonary hygiene, have increased survival in resource-rich countries, and might increase disease prevalence. Before the approval of ERT, only 12.3% of infants with Pompe disease survived to 18 months of age, but in the initial ERT trial, all 18 children treated with ERT before 6 months of age were alive at 18 months[14,15]. ERT, although life-saving, was not definitive and secondary complications of the disease have occurred in most patients receiving ERT alone [16]. In addition, even in the absence of approved therapy, infants with Tay-Sachs disease who historically died at 1.5 years of age now survive for an average of 5 to 6 years of age owing to better supportive therapy [17].

In general, LSDs affect multiple organ systems but have one defining system predominating, such as hypertrophic cardiomyopathy in patients with infantile Pompe disease, CNS deficits in children with neuronal ceroid lipofuscinoses type 2, or hepatosplenomegaly in children with Gaucher disease type I. For Pompe disease and Gaucher disease, the major complications (that is, cardiomyopathy and

hepatosplenomegaly) have been mitigated with approved therapy, and the CNS deficits can be slowed in neuronal ceroid lipofuscinoses type 2 [18]. However, with this increased survival, additional complications have been reported, such as oro-motor deficiencies and diaphragm weakness in Pompe disease[19, 20, 21]. Overall, new treatment regimens have improved the burden of disease, but owing to improved survival, new clinical phenotypes have emerged for some disorders. As treatment becomes available for more LSDs, clinicians should be alert to "secondary phenotypes" that might require additional therapeutic intervention.

[H1] Mechanisms/pathophysiology

[H2] General mechanisms

Lysosomes are responsible for the breakdown and recycling of macromolecules (including carbohydrates, lipids, nucleic acids and proteins) and function as metabolic hubs that control nutrient sensing, amino acid and ion homeostasis and calcium signalling[22-24]. These organelles are constantly in a dynamic state; they fuse with autophagosomes, phagosomes and the plasma membrane, and therefore, have a pivotal role in cell-cell and cell-extracellular matrix communication, response to infection and maintenance of cell homeostasis (Figure 2)[22,23]. In addition, late endosomes-lysosomes tether with other intracellular organelles, such as mitochondrial and the endoplasmic reticulum, without fusing with them, forming functional membrane contact sites. These membrane structures define signalling microdomains that allow for the transfer of lipids and the exchange of metabolites and Ca^{2+} ions between the organelles [24,25,26]. The lipid and protein composition of these contact sites drive their functional characteristics, influencing each of the tethered organelles. For instance, mitochondria-lysosome contact sites modulate mitochondrial fission and lysosomal dynamics through GTP hydrolysis of RAB7[27]. Thus, it is understandable that in the context of an LSD the biochemical properties and in turn the functions of these microdomains may be altered by changes in composition of the endo-lysosomal membranes due to impaired lysosomal activity. [25,26]

As previously mentioned, mutations in genes encoding lysosomal proteins, including lysosomal hydrolases, lysosomal membrane proteins, lipid and ion transporters, enzyme modifiers or activators are the cause of LSDs. Mutations in these genes lead to the aberrant processing and degradation of substances, impaired transport of lipids and metabolites and progressive primary accumulation of non-degraded or partially degraded compounds inside lysosomes. Secondary storage of substrates can also occur in LSDs resulting from defects in non- enzymatic lysosomal proteins e.g. transporters, although the mechanism leading to secondary storage are not fully understood but may be the result of trafficking defects. The biochemical type of storage material differentially affects lysosome function as it relates to specific cellular processes and can lead to cell death, which underlies the variable clinical pathology of LSDs. Cells can only store substrates/metabolites if the cell in question synthesises or ingests these molecules. For example, glycosphingolipid expression is variable in the CNS, so some neuronal populations cannot store these

molecules so are spared, whereas others neurons will store these macromolecules, leading to neuronal damage and death. Circulating monocytes and tissue macrophages (forming the mononuclear phagocytic system) with their phagocytic and endocytic-exocytic capacity are primary affected cells in most LSDs, owing to their role in the phagocytic clearance of cellular debris, apoptotic or necrotic cells, microbes they ingest through phagocytosis and the impaired catabolism of products they themselves synthesise. **[Au: A couple of sentences were added here by different authors; I've added both in and lightly edited this - please check to ensure this is ok ok!]**These cells have a central role in immune responses and inflammatory processes that can be elicited by loss of cell integrity and homeostasis in LSDs[28], indeed, the storage of macromolecule by macrophages in LSDs triggers inflammation that actively contributes to disease progression [29].

Substrate accumulation inside lysosomes can initiate a cascade of secondary effects, ultimately leading to irreversible cellular damage, and organ dysfunction and degeneration. Given the complexity of the clinical presentations and the broad range of accumulated molecules in LSDs, many of which are still unknown, defining pathogenetic pathways potentially common to all disorders is challenging. However, in general terms, several common pathways are dysregulated including deficits in cellular transport and degradation (such as autophagy, endocytosis, phagocytosis and lysosomal exocytosis), calcium homeostasis, oxidative stress, inflammatory and innate immune responses, in addition to cell death pathways (including apoptosis and in some instances necroptosis) [30,31] . In addition, general mechanisms of disease progression include chronic inflammation, including neuroinflammation, which is linked to cellular dysfunction and death, and autoimmune responses to specific self-antigens or accumulated metabolites [28]. Several studies have shown the activation of innate immune cells, such as microglia in the brain and macrophages in the periphery, actively contributes to LSD progression[32-34]. For example, in response to stress signals (i.e. damage released molecular patterns or DAMPs) coming from dying or damaged neurons via pattern recognition receptors, activated microglia release cytokines and chemokines to recruit immune cells at affected sites or to activate neighbouring neural cells in a paracrine/autocrine fashion, hence amplifying the inflammatory response [35]. In line with these findings, anti-inflammatory therapies have shown benefits in some animal models of LSDs[34,36] (such as NPC and Sandhoff disease), and trials of biologic agents that target TNF are in clinical trials in some MPS diseases (see Management). In addition, adaptive immune responses e.g. autoantibody production have been described in some LSDs[37], and the precise pathophysiological role of a dysregulated immune system merits greater investigation, as it is a potentially tractable therapeutic target.

Notably, the common secondary pathways have also been implicated in other, more common adult conditions that are mostly associated with aging, including cancer and neurodegenerative diseases. Moreover, unexpected roles of lysosomal enzymes and their substrates have been demonstrated in cellular

pathways and regulatory networks that go beyond canonical lysosomal degradation or recycling [38,39]. Importantly, this research led to the discovery of parallel mechanisms between rare, paediatric LSDs and common, adult diseases, including age-related neurodegenerative diseases (Parkinson disease (PD) and Alzheimer disease (AD)), in addition to fibrosis and cancer [40-45]. These findings have fostered the development of improved model systems used to evaluate pathogenesis and new treatments, including patient-derived induced pluripotent stem cells (iPSCs) [46-48] differentiated into specific cellular lineages[49,50]. Overall, research on LSDs has sparked the development of therapeutics that may benefit both patients with LSDs or those with other disorders. Below we give some illustrative examples of LSDs where fairly detailed pathogenetic pathways have been elucidated.

[H2] Gaucher disease **[Au: For this section, I accepted all of Sandra's changes and added in some extra details based on Fran's comments (highlighted in yellow) - please check this bit carefully!]**

Gaucher disease, a prototypical LSD, is caused by the deficiency of the lysosomal hydrolase β-glucocerebrosidase (GCase **[Au: I've changed GCase to GBA based on your comments in the Figure 3 legend!]**) encoding gene *GBA*(FIG. 3) [51], leading to the accumulation of the glycosphingolipids glucosylceramide (GlcCer) and glucosylsphingosine (GlcSph). GlcCer and GlcSph accumulate in mononuclear phagocytes (owing to their increased burden of glycosphingolipid catabolism due to the phagocytic activity of these cells **[Au: OK?]ok with added edits**), primarily macrophages, which have a foamy appearance and are referred to as Gaucher cells [51,52]. Investigations into the chronic inflammation and hematologic abnormalities in patient-derived primary macrophages[53] have identified defects in macroautophagy and the degradation, but not initial uptake of apoptotic or necrotic cells (efferocytosis) [49], likely as a result of glycosphingolipid storage[54,55]. In acute and chronic Gaucher disease type II and III, which are neuropathic, microglia activation and neuroinflammation have been associated with neuronal dysfunction and neuronal loss in the brain and spinal cord [56,57]. The mechanism of neuronal loss and inflammation in murine models of Gaucher disease **[Au:OK?]yes** is deregulated processing and release of pro-inflammatory cytokines (i.e. IL-1α, IL-1β, TNF-α, IL-6, Cxcl-10 and type I interferon[58,59]; altered chemotaxis of inflammatory and immune cells, and production of reactive oxygen species.[60-65] This pathogenetic cascade has been studied in a conditional knockout model of Gaucher disease (Gbaflox/flox nestin-Cre mouse), which suggests that neuroinflammation is elicited by DAMPs released from dying neuron, astrocytes and oligodendrocytes [58,66,67]. These effectors promote microglial pro-inflammatory activity, which amplify their neurotoxic process ultimately leading to neurodegeneration[35]. In the same Gaucher mouse model, the upregulation of receptor-interacting protein kinases (RIPK1 and RIPK3), coupled to necrotic cell death and inflammasome activation, have also been implicated in neurodegeneration.[68] In addition, the chronic inflammation in experimental and clinical Gaucher disease

could be due to the production of complement-activating GlcCer-specific IgG autoantibodies, which have been proposed to drive a cycle of GlcCer and GlcSph accumulation and complement activation[62].

Font change occurs here! Natural history and population genetic studies have revealed that patients with type 1 Gaucher disease or carriers of *GBA1* mutations disease have an increased risk of Parkinson disease, or Lewy body disorders[45,69,70]. These neurodegenerative conditions are characterized by the presence of insoluble, oligomeric or fibrillar α-synuclein inclusions in neurons of the substantia nigra, the hippocampus and the cerebral cortex [55,71]. Interestingly, an inverse relationship between the levels of GBA and α-synuclein aggregates has been demonstrated experimentally in model systems (cells and mice), but the mode of interaction between these molecules and their contribution to the development of synucleinopathies, have not been fully elucidated[55,72,73]. In addition, GlcCer directly controls a reversible change in α-synuclein that promotes its aggregation and toxicity, opening the possibility of therapeutic intervention by glycosphingolipid reducing agents[74]. Additional work is needed to fully understand the connection between *GBA* mutations, particularly in heterozygous individuals, and predisposition to Parkinson disease. Significantly, a recent publication implicates lysosomal dysfunction as the link between LSDs and Parkinson disease (not specifically *GBA* mutations) as heterozygous mutations in 54 of the 70 LSDs are over represented in patients with sporadic Parkinson disease [75].

[H2] GM1-gangliosidosis

GM1 gangliosidosis is a generalized CNS disease (that is, affecting several brain regions) caused by deficiency of lysosomal β-galactosidase, which cleaves the sialyl-glycosphingolipid GM1-ganglioside (GM1) [76]. GM1 is abundant in neuronal membranes, and is the only ganglioside shown to bind calcium and modulate calcium flux across membranes[77-79]. GM1 accumulation in the brain and spinal cord of patients with GM1 gangliosidosis promotes generalized neurodegeneration, and similarly, $\beta\text{-}Gal^{-/-}$ mice undergo progressive neuronal cell death followed by widespread neuroinflammation [33,80]. Mechanistically, the neuronopathic phenotype is caused directly by GM1 accumulation in neurons, as this molecule abnormally accumulates in the endoplasmic reticulum membranes in $\beta\text{-}Gal^{-/-}$ neurons, causing endoplasmic reticulum calcium depletion, activation of the unfolded protein response (UPR) which induces apoptosis [81]. In addition, GM1 accumulates at membrane contact sites between the endoplasmic reticulum and mitochondria, known as mitochondria-associated endoplasmic reticulum membranes (FIG. 3), and specifically in glycosphingolipid-enriched or raft-like microdomains (GEMs), which affect mitochondrial function [82,26]. At GEMs, the interaction of GM1 with the endoplasmic reticulum calcium channel inositol trisphosphate receptor 1 (IP3R1) promotes abnormal calcium flux from the endoplasmic reticulum to the mitochondria, leading to endoplasmic reticulum calcium depletion **[Au:OK?ok]**, mitochondrial calcium overload and activation of mitochondria-mediated apoptosis [82]. This combined pathogenetic cascade,

directly downstream of increased GM1 levels, explains, at least in part, the complex CNS phenotype of GM1-gangliosidosis.

It is currently unknown whether β-GAL mutations or polymorphisms or β-GAL haploinsufficiency could be risk factors for AD. However, several studies have demonstrated the occurrence of amyloid-β (Aβ)-generating secretases in GM1-containing lipid rafts, and GM1-bound Aβ peptides with altered confirmation have been discovered in an autopsied brain of a patient with early-stage AD. It has been hypothesized that Aβ bound to GM1 functions as an endogenous seed for amyloid fibril deposition in AD brains [83-85]. Interestingly, GM1 is differentially distributed in the hippocampal grey matter of patients with Alzheimer disease compared with normal **[Au: 'healthy'?yes]** control individuals [86,87].

[H2] Sialidosis

Sialidosis is a rare neurodegenerative LSD that is caused by deficiency neuraminidase 1 (NEU1), which cleaves sialic acid residues. Although the full range of NEU1 substrates is not yet known, the enzyme preferentially cleaves soluble sialyl-oligosaccharides or glycopeptides, which are typically excreted in body fluids, but accumulate in lysosomes in NEU1-deficient cells **[Au:OK? yes]** [88].In addition, NEU1 can also cleave sialic acids on membrane glycoproteins, and, in doing so, alters their biochemical properties, which then negatively affects cell-cell adhesion, cell migration, receptor-ligand recognition and signaling, and antigen presentation [88,89]. *Neu1*$^{-/-}$ mice have phenotypes that resemble those of type 2 sialidosis, and manifest with neurodegeneration, muscle atrophy and splenomegaly, among other features[90]. Searching for the molecular cause(s) of these phenotypes identified NEU1 as a negative regulator of calcium-driven lysosomal exocytosis [41-43,91,92]. NEU1 regulates lysosomal exocytosis by cleaving the sialic acids from LAMP1, a mediator of endo-lysosome docking at the plasma membrane [91,93]. Different cell types, including neurons, with absent or reduced NEU1 activity have increased LAMP1-positive endo-lysosome docking and excessive exocytosis of hydrolytic enzymes and other endo-lysosomal contents, including exosomes [91] (FIG. 3). Increased lysosomal exocytosis is at the basis of sialidosis pathogenesis in the mouse model and likely in patients, but the molecular effectors downstream of excessive lysosomal exocytosis varies between cells with different physiological functions, and likely with a different composition of lysosomal contents. For instance, in the bone marrow excessive exocytosis of lysosomal proteases into the bone marrow extracellular fluid causes the loss of retention of hematopoietic progenitor cells within the bone marrow niche, their mobilization to peripheral blood and their increased numbers in the spleen, and consequently, time-dependent splenic extramedullary hematopoiesis and spleen enlargement [91]. In muscle, increased exocytosis of matrix metalloproteases and cathepsins from connective tissue fibroblasts, and increased synthesis and deposition of collagens and other extracellular matrix (ECM) components triggers progressive expansion of the connective tissue that gradually invades the muscle bed, leading to myofiber fragmentation and muscle degeneration [42]. These findings have shifted the focus of sialidosis research to the effect of NEU1-regulated

exocytosis of hydrolytic enzymes and exosomes on the extracellular microenvironment, which has important ramifications for the biology of other conditions, including cancer [43] (FIG. 3).

Font change. Interestingly, *Neu1*$^{-/-}$ mice have progressive β-amyloid deposition in the brain, particularly in the CA3 region of the hippocampus, which resembles plaque formation in AD patients and animal models for their location and composition[41]. Amyloid precursor protein-positive aggregate deposition occurs through two concomitant events: lysosomal accumulation and aberrant processing of an over-sialylated amyloid precursor protein (which is a substrate of NEU1) and, increased lysosomal exocytosis of toxic Aβ-42 polymers into the brain cerebrospinal and interstitial fluid[41]. Thus, *Neu1*$^{-/-}$ mice may be a spontaneously occurring model of Alzheimer disease, raising the intriguing possibility that NEU1 loss of function may contribute to the development of sporadic Alzheimer disease via altered lysosomal exocytosis in the central nervous system.

[H2] Niemann-Pick type C

Defects in non-enzymatic lysosomal proteins can also cause LSDs, such as Niemann-Pick disease type C (NPC). NPC is unusual as two apparently unrelated genes cause the same disease suggesting that the proteins they encode function in a common cellular pathway. NPC1 is caused by mutations in *NPC1* that encodes a multi-pass membrane protein (NPC1) residing in the limiting lysosomal membrane, whereas NPC2 is caused by mutation in *NPC2* that encodes a soluble cholesterol binding protein (NPC2)[94]. The majority of clinical cases of NPC (95%) have mutations in *NPC1* and present in infancy or childhood with a progressive neurodegenerative disorder, although adult onset cases occur and are likely under diagnosed [94]. NPC1 might have a role in the transport of sphingosine out of lysosomes and accordingly, the pathogenetic cascade in NPC is initiated by increased sphingosine storage that, in turn, via an as yet unknown mechanism, reduces calcium levels in the lysosome [95]. Reduced calcium levels in lysosomes[95-98] means that insufficient calcium is released from the lysosome so is unavailable for use by calcium-dependent proteins involved in lysosome fusion and trafficking, leading to substantially impaired late endosome-lysosome fusion[99]. Consequently, lipids, including LDL-derived cholesterol and sphingolipids (such as glycosphingolipids and sphingomyelin) are stored in many cell types, which can further contribute to the pathogenic cascade but remain incompletely understood(FIG. 2 and 3). The consequences of lipid storage in NPC include for example mitochondrial dysfunction and activation of the innate immune system leading to inflammation. The major issues to be resolved in the NPC field are how and why NPC1 and NPC2 cooperate in a common cellular pathway and whether the NPC1 protein is a cholesterol regulated transporter or a dedicated cholesterol transporter, as many view it to be. Based on homology to bacterial orthologues it is likely that NPC1 is involved in multi-substrate transport [100-102].

Font! Dementia is a common symptom in patients with NPC, with the histopathology of the brain post-mortem sharing many histopathological features classically associated with late onset Alzheimer

disease. The links between these two diseases suggest that the cell biology of NPC accelerates amyloid precursor protein processing and tau phosphorylation defects typically associated with aging in the general population [103].

[H2] LROs

LROs are cell-specific intracellular vesicles that are found in specialized cell types, for example melanocytes, lymphocytes and in platelets, among others (Fig 4). LROs share features with endo-lysosomes, such as low pH, the presence of specific membrane proteins and a similar pathway of formation (FIG. 4), but are distinct in function, morphology, and their cargoes that impart their physiologic properties[104,105]. Genetic defects that affect the formation, maturation and secretion of LROs can affect their functions in either one LRO-containing cell type or multiple cell types, resulting in a variety of clinical features referred to as LRO disorders (TABLE 1). Cells from patients with LRO disorders (or LRO disorder mouse, fly or zebrafish models) are important tools for the investigation of LRO biogenesis, vesicular trafficking, membrane remodelling and mechanisms of secretion.

[H1] Diagnosis, screening and prevention

As previously mentioned LSDs are monogenic disorders and the vast majority are inherited in an autosomal recessive manner. Exceptions to this inheritance pattern include MPS II (X-linked recessive), CLN4 (dominant and recessive forms), Fabry disease (X-linked, although some females might have a later onset disease that does not usually involve the kidney) and Danon disease (X-linked, in which females have later onset than males, and might have a milder phenotype that does not include skeletal myopathy or intellectual disability (TABLE 1).

[H2] Clinical features

[H3] Enzyme deficiency disorders. The classical LSDs (that is, the enzyme deficiency disorders) can be grouped according to the stored material and include sphingolipidoses, mucopolysaccharidoses, glycogen storage disease and glycoproteinoses (TABLE 1). The age of symptom onset is usually determined by the amount of residual enzyme activity, which is governed by the specific genetic mutations. These disorders are most often multisystem disorders that have multiple subtypes, and can have symptomatic onset from the prenatal period to adulthood.

The majority of enzyme deficiency disorders involve CNS dysfunction, often presenting with neurological symptoms. **[Au: moved to 'Diagnosis', below, as discussed OK]** In adults, the onset of disease can be subtle, and dysarthria (difficulty with speech), ataxia, weakness, or abnormal movements are common

presenting features and are easily confused with symptoms of more common adult-onset neurodegenerative disorders. Psychosis or depression can also be a presenting feature in adult-onset disorders, such as late onset Tay-Sachs disease, and can delay diagnosis by years to decades [106]. Substrate storage in specific organ systems is characteristic of some enzyme deficiency disorders, such as storage in the kidney in Fabry disease, the heart in Pompe disease, or the mononuclear phagocyte system in Gaucher disease. galactosialidosis and sialidosis, and sialyloligosaccharidurial **[Au: please define - the presence of sialic acid and galactosidase in the urine? This might fit better in diagnosis section; I would remove it here. [Au: I've left this here for now; where should we move this text - would this work on line 506?]]** in sialidosis and galactosialidosis, and the resulting organ or system-specific manifestations should facilitate prompt diagnosis of these disorders. Yes remove as does not fit well here . Similarly, common constellations of findings such as hepatosplenomegaly, coarsening facial features, and joint contractures (with or without corneal clouding) should suggest a mucopolysaccharidosis. However, more subtle presentations such as mild muscle weakness in an adult (which can occur in Pompe disease) or a clinical sign with more common etiologies such as proteinuria, diminished renal function, or cardiac hypertrophy (which can occur in Fabry disease) can make diagnosis more challenging.

[H2] Disorders of post-translational modification.

Disorders of post-translational modification include multiple sulfatase deficiency and the mucolipidoses II and III, and result from mutations in genes that have a role in biochemically modifying lysosomal hydrolases. Therefore, the clinical manifestations of these disorders are more generalized than other forms of LSDs, and have overlapping features with disorders caused by defects in single lysosomal hydrolases (TABLE 1). For example, multiple sulfatase deficiency is caused by mutations in *SUMF1*, which encodes formylglycine-generating enzyme (FGE). FGE modifies sulfatases (a class of lysosomal enzymes) which include the enzymes that degrade mucopolysaccharides in MPS II, MPSIIIA, MPSIIID, MPSIVA and MPSVI. As a result, the clinical manifestations of multiple sulfatase deficiency overlaps with the MPS disorders making diagnosis challenging. Similarly, mucolipidosis II and III are caused by mutations in *GNPTAB* and *GNPTG*, respectively, encoding the subunits of GlcNac-1-phosphotransferase , which tags lysosomal hydrolases with mannose-6-phosphate for targeting to the lysosome. As a result, hydrolases that require mannose-6-phosphate tagging (which includes degradative enzymes for most sphingolipids, mucopolysaccharides and glycoproteins) are not trafficked to the lysosome and are secreted into the extracellular space. This process leads to the accumulation of multiple complex substrates in lysosomes, which leads to disorders with severe phenotypes that share clinical features with the sphingolipidoses, glycoproteinoses and mucopolysaccharidoses, but, most notably the MPS disorders.

[H3] Disorders of integral membrane proteins. Like the enzyme deficiency disorders most disorders of integral membrane proteins affect the CNS, and common symptoms include intellectual disability, ataxia,

seizures and spasticity. Also similar to the enzyme deficiency disorders, disorders of integral membrane proteins can present during infancy, childhood and adulthood. With the exception of NPC and cystinosis, most integral membrane protein disorders are rare in the general population. Cystinosis - which does not primarily affect the CNS - is characterized by proximal tubular dysfunction due to cystine accumulation, which leads to renal Fanconi syndrome and renal failure in >90% of patients [107]. The prompt diagnosis and treatment with cysteamine prevents renal failure. However, cystinosis is a systemic disorder other symptoms include photophobia and visual impairment due to corneal deposition of cystine crystals (which can be treated effectively with cysteamine eye drops), endocrine abnormalities (such as hypothyroidism and growth hormone deficiency), and skeletal deformities (including scoliosis, stress fractures and joint pain). Even with cysteamine therapy, patients with cystinosis can also develop a secondary phenotype of progressive myopathy and muscle weakness, dysarthria, and swallowing difficulties. NPC type 1 or 2 should be suspected in a child with isolated splenomegaly or neonatal liver disease, as patients can have no other clinical findings for years before developing the characteristic supranuclear gaze palsy followed by intellectual decline. Neuropsychiatric symptoms and cognitive decline can predominate in NPC with onset in adolescence and adulthood, resulting in long delays in diagnosis [108,109].

[H3] The neuronal ceroid lipofuscinoses. The neuronal ceroid lipofuscinoses involve the CNS, and were initially classified based on age of onset[9] (TABLE 1). More recently, the classification was updated to include the mutation as well as the age of onset (for example, CLN3 disease, juvenile onset). Symptom progression is variable [10]; some disorders present initially with progressive visual loss (for example CLN3 disease, juvenile onset)[110], followed by mental decline and seizures[10]. Indeed, most patients with CLN3 disease are diagnosed by ophthalmologists owing to the early visual loss with characteristic retinal pathologies[110]. Other NCLs present with developmental delay (for example CLN2 disease, late infantile onset), with visual loss occurring at later stages[111]. In general, the NCLs involve progressive movement disorders, epilepsy, dementia and early death.

[H3] Disorders of LROs. The most notable LRO disorders include the Hermansky-Pudlak, Griscelli, and Chediak-Higashi syndromes, which are all characterized by hypopigmentation (owing to a melanosome defect) and prolonged bleeding (owing to platelet delta granule defect)[104]. The classic form of Chediak-Higashi syndrome also includes recurrent life-threatening infections due to immunodeficiency and 85% develop a life-threatening hyperinflammatory condition (hemophagocytic lymphohistiocytosis). Untreated patients die, usually of overwhelming bacterial infection, in infancy or early childhood. [104]. Most LRO disorders manifest during infancy, with the exception of Chediak-Higashi disease, which has juvenile and adult-onset forms. Hematopoietic stem cell transplantation (HSCT) facilitates improved survival of patients

with classic Chediak-Higashi syndrome into adulthood, although a secondary phenotype similar to the adult-onset form subsequently emerges, including tremor, ataxia, peripheral neuropathy, and cognitive decline that is life-limiting [112].

[H2] Diagnosis

The diagnosis of most LSDs is straightforward if they are suspected based on clinical presentation; however, a diagnosis of LSDs is often not clinically suspected owing to the general symptoms of these disorders and their rarity. **[Au: moved to here from 'Enzyme deficiency disorders' as discussed]** Any infant or child aged between 6 months and 10 years of age who initially has a period of normal development, but plateaus and loses previously attained skills should be considered to have an LSD until proven otherwise, irrespective of whether the LSD proves to be an enzyme deficiency or caused by a different LSD-causing mechanism. Diagnostic work up can be initiated with screening for a class of disorders that have similar clinical presentations; one example of such a screen is the detection of glycosaminoglycans in the urine of patients with mucopolysaccharidosis. Definitive diagnostic testing for specific enzyme deficiency disorders includes the evaluation of lysosomal enzyme levels in peripheral blood leukocytes using artificially-synthesized fluorogenic substrates specific for each enzyme. [113] Enzyme levels are expressed as amount of substrate cleaved per milligram of total protein per unit time, and are compared with a normal range[113]. When enzyme levels fall below the normal range, a diagnosis of a specific LSD can be confirmed using genetic testing, to identify mutations in the gene encoding the deficient enzyme. However, enzyme assays are performed *in vitro* on artificial substrates, and might not accurately represent enzyme activity against natural substrates *in vivo*. For some enzymes, like hexosaminidase A, the testing can also be performed on patient serum or plasma. Indeed, such testing formed the basis of carrier detection of infantile Tay-Sachs disease in the Ashkenazi Jewish population (see Carrier screening, below)[114]. Unfortunately, carrier testing in serum or plasma is not reliable for many lysosomal disorders.

Genetic testing to identify specific mutations can be performed using DNA sequencing, and can complement the enzyme studies and suggest prognosis if those specific mutations have been demonstrated in other patients. However, if patients do not have parental consanguinity or are members of a high-risk population, they are generally compound heterozygotes, which makes phenotype-genotype correlation difficult. Some LSDs, such as disorders of integral membrane proteins, most of the neuronal ceroid lipofuscinoses, and the LRO disorders can only be diagnosed by direct gene sequencing. If identified mutations have been previously reported as pathogenetic or disease-causing, a diagnosis of LSDs can be confirmed. However, if new variants of undetermined significance are found, then the results are tentative and must be correlated with the clinical phenotype.

Despite available diagnostic testing for LSDs, the key to diagnosis, particularly in patients after infancy, is considering an LSD diagnosis in the first place. In adults, several more common disorders can present with symptoms similar to LSDs, including ataxia, myoclonus, dementia or psychiatric symptoms, and many clinicians are unaware that LSDs can present in adulthood. Juveniles might present with plateauing of academic skills with subsequent regression as well as falling, increased difficulty walking and talking, visual difficulties or behavioural changes; all of which are general symptoms with a broad differential diagnosis.

Diagnosis of LSDs must be commenced early for effective treatment, a target that is often not met. In one study of children with late-infantile and juvenile type II GM1 gangliosidosis, the average time to diagnosis from the time of symptom onset was 24 months and 9.8 years respectively [115]. In infants and toddlers, development is somewhat variable and accordingly, paediatricians will often monitor late-infantile patients for several months until skills are clearly outside the range of normal development, or a child is actually losing skills, before referring the child to a neurologist. Similarly, the neurologist might also take several months to complete diagnostic testing before arriving at a diagnosis. For juveniles who develop typically up to 4 to 5 years of age, the process of observation through the plateau phase and consideration of more common problems including attention deficit/hyperactivity disorder and autism spectrum disorders might take even longer. Type II GM1 gangliosidosis includes progressive brain atrophy implying that delays in diagnosis would result in less effective or ineffective therapy. Most paediatricians and many neurologists are not aware that LSDs can present in juveniles, and cranial imaging could initially show only non-specific findings. The increasing use of next generation sequencing is expected to aid in more timely diagnosis since practitioners would not have to have a specific neurologic diagnosis in mind in order to initiate diagnostic testing. This will also lead to identification of new genetic variants of uncertain significance and may also expand the clinical phenotype of individual LSDs as new genetic variants are described[116].

[H2] Screening and Prevention

[H3] Carrier Screening. Tay-Sachs disease is the prototypical LSD for population-based carrier screening, as this disorder has a carrier frequency of 1 in 27 in Ashkenazi Jewish individuals[117]. Community screening was commenced in the early 1970's in the United States and consisted of plasma-based enzyme assay to identify couples who were both Tay-Sachs disease carriers and, therefore, have a 25% risk of having a child with infantile Tay-Sachs disease. With this information couples, could undergo amniocentesis or chorionic villus sampling to identify affected foetuses and consider pregnancy termination, or could choose to adopt a child. Couples can now undergo IVF followed by pre-implantation diagnosis to identify unaffected embryos for uterine implantation. Screening within the Ashkenazi Jewish community decreased the number of children born with Tay-Sachs disease in the United States from 60 per year before 1970 to 3–5 cases per year to by 1983 (a 90% reduction in the incidence within this population)[114], and resulted in the birth of ~2,000 healthy infants from at-risk couples, who would otherwise not have attempted a pregnancy. The