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Disease models for the development of therapies for lysosomal storage diseases

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Abstract

Lysosomal storage diseases (LSDs) are a group of rare diseases in which the function of the lysosome is disrupted by the accumulation of macromolecules. The complexity underlying the pathogenesis of LSDs and the small, often pediatric, population of patients make the development of therapies for these diseases challenging. Current treatments are only available for a small subset of LSDs and have not been effective at treating neurological symptoms. Disease-relevant cellular and animal models with high clinical predictability are critical for the discovery and development of new treatments for LSDs. In this paper, we review how LSD patient primary cells and induced pluripotent stem cell (iPSC)–derived cellular models are providing novel assay systems in which phenotypes are more similar to those of the human LSD physiology. Furthermore, larger animal disease models are providing additional tools for evaluation of the efficacy of drug candidates. Early predictors of efficacy and better understanding of disease biology can significantly affect the translational process by focusing efforts on those therapies with the higher probability of success, thus decreasing overall time and cost spent in clinical development and increasing the overall positive outcomes in clinical trials.

Keywords

lysosomal storage diseases; therapeutic development; cell-based disease model; induced pluripotent stem cells; animal models

Introduction

Lysosomal storage diseases (LSDs) are a group of approximately 50 genetic disorders caused by mutations in genes encoding proteins necessary for lysosomal function, in most cases, enzymes involved in the cellular degradation and trafficking of lipids and other macromolecules. Individual LSDs are rare but together they are a relatively common group

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Conflicts of interest

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of diseases with a combined prevalence of approximately 1:8000 live births.[1–3] LSDs were originally named and categorized by their clinical presentations or the name of the physician who first identified them, such as Batten, Gaucher, and Niemann–Pick diseases.[4] Advances in the understanding of the causative mutated genes and affected cellular molecules in the lysosomes have now allowed for the classification of LSDs into 48 individual diseases, and some of them have been renamed according to the nature of the accumulated products in the lysosomes. The current naming of the LSDs as established by the Lysosomal Disease Network is shown in Table 1.

A common feature of LSDs is the accumulation of lipids, glycoproteins, and other macromolecules in lysosomes as a consequence of the functional deficiency of a specific lysosomal protein in the affected cells (Table 1). The accumulation of these molecules normally causes an enlargement of the lysosomes and reduces the supply of macromolecules, or downstream products, for use in critical cellular functions, such as molecular biosynthesis and energy metabolism.[5, 6] Although clinical manifestations and onset of disease vary significantly among the different LSDs, hepatomegaly and splenomegaly are two of the most common symptoms,[4, 6] and in more than half of LSDs, central nervous system (CNS) symptoms accompanied by neuronal degeneration occur.[7]

For each LSD, single or multiple mutations in the gene causing the disease have been identified. Often, many of the mutations involve a single base pair change that alters one amino acid in the encoded protein. These missense mutations either reduce the biological activity of the enzyme/protein or impair translocation to the lysosome, causing premature protein degradation. In some LSDs, as in the case of Gaucher disease, there are a few predominant mutations, such as the N370S (70% of patients) and L444P (10% of patients) mutations in β-glucocerebrosidase (GCase).[8] In other LSDs, such as Niemann–Pick disease type C (NPC), the mutations identified in the *NPC1* or *NPC2* genes are more diverse, making the genetic mutation variability in the patient population greater.[9] Although the genetic causes of LSDs are known, the biology underlying the clinical symptoms of each disease is complex and, in most cases, is not fully understood, especially the neurodegenerative symptoms. In addition, therapeutic targets for LSDs have, so far, not been very tractable using traditional drug discovery approaches.

Current approved treatments for LSDs are based on the replacement of the mutated protein with native protein in order to restore its wild-type function (enzyme replacement therapy, ERT) or on the inhibition of the enzymes upstream of the mutated causative protein in order to reduce production of accumulating substrates (substrate reduction therapy, SRT). To date, there have been successful drug approvals for the LSDs with higher prevalence, regulatory precedent, and a proven technology platform, making commercial investment more viable because of the lower risk and higher return on investment. The U.S. Orphan Drug Act was enacted in 1983 to stimulate investment into therapeutic development for rare diseases through financial incentives. Seventeen drugs for LSDs received U.S. Food and Drug Administration (FDA) approval between 1983 and 2015[10, 11] but only cover nine different LSDs, highlighting the fact that the majority of LSDs still do not have an approved therapy. An ERT is available for each one of the nine diseases for which therapies have been approved: Gaucher disease, Fabry disease, mucopolysaccharidoses I (MPS I), MPS II

(Hunter syndrome), MPS IVA (Morquio A syndrome), MPS VI, Pompe disease, and lysosomal acid lipase deficiency (LAL-D). Two diseases have multiple drug approvals: Gaucher disease has six drug approvals and cystinosis has three. In the European Union, 15 products for LSDs were authorized for treatment between 1995 to 2015, most of them being the same products approved by the U.S. FDA.[12, 13] A drawback with the current ERT therapies for LSDs is that patients with neuronal LSD manifestations cannot be treated because recombinant enzymes cannot cross the blood–brain barrier.[14] Therefore, effective treatments for LSDs are still greatly needed. There is now ample evidence that disruption of lysosomal functions are also involved in the pathogenesis of a variety of age-related diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS), and multiple sclerosis (MS).[15, 16] For this reason, interest in the development of treatments for LSDs has increased because common underlying mechanisms between lysosomal and neurodegenerative diseases may lead to the development of therapeutics for diseases with larger populations of patients.[17, 18]

The success rate of the drug discovery process is generally very low, mostly because of the lack of efficacy of drug candidates in clinical trials. An analysis of phase II failures for new small-molecule drugs and new indications of existing drugs between 2008 and 2011 found that 51% of failures were because of insufficient efficacy in the clinic, 29% because of strategic decisions, and 19% because of preclinical or clinical safety concerns.[19] When examining these failures by therapeutic areas, 21% and 16% of the failures were in metabolic and neurological diseases, respectively.[20] While some of these drug failures in the clinic can be attributed to clinical trial designs that did not have clear objectives, measurable and quantitative endpoints, or defined target patient populations, one of the largest hurdles to a higher success rate in drug development has been the low clinical predictability of the existing preclinical disease animal models. More often than not, positive efficacy results observed in preclinical animal models do not reproduce in clinical trials. The use of disease animal models for efficacy and safety studies in therapeutic development is based on the principles of evolutionary conservation between species, including conservation of pathogenic disease mechanisms.[21] However, a lack of clear understanding of the physiology of the disease in humans versus animal species used in the pre-clinical studies often leads to incomplete matching of phenotypes, especially in genetically engineered mice. Preclinical disease models that are more predictive of the efficacy of a drug candidate in humans would significantly reduce the time and cost of development, identify better-responding patient populations, and increase the overall positive outcomes in phase II and III clinical trials.[22, 23]

Highly predictive *in vitro* and *in vivo* preclinical disease models are especially critical for rare diseases, including LSDs, where the early onset and small number of patients make the design of clinical studies very challenging.[24] Predictive *in vitro* cell-based assays with human disease cells, for example, cells derived from patient induced pluripotent stem cells (iPSCs), can provide critical efficacy data to help guide clinical development. These patient iPSC-derived cells are being produced as alternative preclinical models to aid in understanding the pathological pathways causing LSDs and to serve as predictive assay platforms for drug development.[25] These cell-based disease models contribute toward the identification of new targets and biomarkers, and for screening of small-molecule libraries for drug discovery. For *in vivo* testing of potential therapeutic candidates, large animal

models, such as cats and dogs with spontaneous natural mutations or pigs with genetically engineered mutations, hold promise as models for improving the predictability of human disease phenotypes and efficacy.[23] These animal models have supported LSD drug approvals by contributing to a better understanding of the natural history of disease progression, defining a drug candidate's pharmacokinetic and pharmacodynamic relationship and defining clinical endpoints.

As mentioned above, patient iPSC-derived cells and animal models are especially important for rare disease, such as LSDs, because the limited number of patients restricts the number of therapeutic candidates to be tested in clinical trials. Therefore, the development of therapies for LSDs depends even more on the predictability of preclinical models for successful drug development. It would certainly be very valuable to be able to decide prior to a human clinical trial which molecule has the lowest risk and highest probability of success in order to move forward in a small rare-disease population.[23, 24]

LSD pathogenesis

Lysosomes are cellular organelles whose primary function is to break down macromolecules for export or recycling, and they are now thought to play a significant role in the regulation and maintenance of cellular homeostasis.[5, 7] There is a dynamic interaction between the endosomal and lysosomal system involving the secretory–endosomal–autophagic–lysosomal–exocytic (SEALE) network, which is needed for proper processing of cellular macromolecules.[15] Many cellular events regulate lysosome function, including pH, calcium homeostasis, nutrient surveillance through amino acid sensing,[26,27] and starvation response through mTOR localization and modulation.[28, 29] Activation of lysosomal function eliminates damaged proteins through chaperone-mediated autophagy, and misfolded proteins and aggregates through macroautophagy,[30] maintains mitochondrial homeostasis through mitophagy, induces phagocytosis and receptor-mediated endocytosis, [31, 32] and regulates its own biogenesis function upon activation of the transcription factor EB (TFEB)[33–35] and transcription factor E3 (TFE3).[36] TFEB has been found to regulate a specific gene network of approximately 500 genes involved in lysosomal biogenesis and autophagy, referred to as the coordinated lysosomal expression and regulation (CLEAR) network.[5] LSD mutations have been shown to downregulate TFEB, leading to the deregulation of lysomal formation and autophagy.[37, 38]

Lysosome dysfunction is the direct cause of the pathogenesis of LSDs. In LSDs, mutations in lysosomal hydrolases or transporters result in the accumulation of specific macromolecules (Table 1), leading to progressive reduction in the capacity of the lysosome to respond to cellular queues and normal processing, which, in turn, leads to secondary changes in cellular events, such as impairment in autophagy, mitochondrial dysfunction, inflammation, and cell and tissue death.[5] Even though the defective proteins in LSDs are found in multiple cell and tissue types, there is selective vulnerability in the extent to which these lysosomal defects affect the functioning and viability of each cell type. For example, a reduction in GCase activity can be observed in Gaucher disease patient fibroblasts and macrophages, but accumulation of glucosylceramides is only observed in macrophages.[39] Fibroblasts with reduced GCase activity appear to be able to maintain cellular lipid

homeostasis. Macrophages are the major cells that play an important role in Gaucher disease symptoms affecting the liver, spleen, bone marrow, and bone remodeling.[8] In NPC disease, cholesterol accumulation can be observed in all patient cells, including skin fibroblasts, liver, and neuronal cells.[40] However, it is the viability of the neuronal cerebellum Purkinje cells that is affected to a greater extent by the cholesterol trafficking defect, leading to progressive neurodegeneration.[41]

The reasons for the differences observed in the severity in LSD pathogenesis among different cell types are not yet clearly understood. However, the genetic defects leading to dysfunctional lysosomal activity have identified some common lysosomal functions that are disrupted upon storage, independent of the type of storage material. Targeting these common lysosomal functions could provide new avenues for drug discovery that may be more broadly applicable across LSDs, particularly neurodegenerative diseases.[5] For example, it has been reported that δ-tocopherol normalizes the cellular phenotypes of several LSDs, including Niemann-Pick disease types A and C (NPA and NPC), Batten (Ceroid Lipofuscinosis Neuronal 2, CLN2), Fabry, Farber, Sanfilippo type B (MPS type IIIB, MPS IIIB), and Tay–Sachs diseases.[42] Since these LSDs do not accumulate the same storage materials, the ability of δ-tocopherol to enhance storage clearance in patient-derived disease cells suggests a common mechanism leading to the enhancement of lysosomal function and exocytosis. It has also been found that overexpression of TFEB stimulates exocytosis and enhances clearance of storage materials. Therefore, TFEB activation could also improve lysosomal function in many LSDs and improve the phenotype for these diseases. Decreased lysosomal storage was found in sulfatase deficiency (MSD), MPS IIIA, and Pompe cell lines overexpressing TFEB.[43] In neurons, autophagy has been well established as essential to cell survival. In addition to LSDs, a group of classical aggregate-dependent neurodegenerative diseases, including Alzheimer's disease, Huntington's disease, Parkinson's disease, and ALS, are currently perceived to be caused by a loss of cellular macroautophagy capacity and may also be related by a failure of lysosomal function.[44] It is therefore very plausible that interventions developed for the treatment of LSDs may also ameliorate these neurodegenerative disorders.[45, 46] Several reports already have indicated connections between NPC and Alzheimer's disease[47] and between Gaucher disease and Parkinson's disease.[48]

Development of therapeutics for LSDs

In order for a drug to be approved by regulatory agencies, it must meet statutory legal requirements of clinical effectiveness established by substantial evidence as determined by adequate and well-controlled clinical investigations.[49] In rare diseases, with the small patient numbers and often pediatric age range of the disease populations, it can be difficult to standardize endpoint measurements and measure statistically significant differences between the drug-treated and control group.[24] Therefore, it is critical to acquire as much information as possible about the safety and efficacy of a new therapy in preclinical models to demonstrate feasibility prior to human clinical testing. Both *in vitro* cell-based and *in vivo* animal models have been used to study disease mechanisms and develop therapies for LSDs. Current therapies for LSDs have focused on trying to correct the primary underlying genetic protein defect, with the overall goal being to reduce substrate accumulation in lysosomes,

either by increasing the amount of active enzyme (ERT) or by inhibiting the production of substrates (SRT).[10, 12, 50, 51] In the development of ERTs, initial studies in patient fibroblasts of human MPS led to the understanding of how lysosomal enzymes are trafficked to the lysosome and of the underlying concept of enzyme complementation to restore function.[52] However, since the implementation of ERT in the early 1990s, limitations of protein replacement therapies have been recognized. For example, the enzymes do not reach all disease-relevant cells, tissues, and organs, and in particular, as currently dosed by intravenous (IV) administration, the enzymes do not have an effect on CNS symptoms because they cannot penetrate the blood–brain barrier.[14, 53, 54] Furthermore, the efficacy and tolerability of ERTs can be limited by immunogenicity and the formation of neutralizing antibodies.[55] ERT is also expensive because of the weekly IV administrations and the high costs associated with manufacturing of recombinant proteins.[56, 57] For the first ERT, developed for Gaucher disease, the management of type I disease, which has no neuronal involvement, has been effective, but it has not been as effective for the treatment of type II or type III disease, which have a neuronal phenotype.[58, 59] In MPS I, while ERT improves symptoms related to respiratory, cardiac hypertrophy, joint movement, and walking, it has little or no effect on cardiac valves, the skeletal system, or CNS.[60–62] In other approved ERT therapies, the effectiveness has also been found to vary, dependent on the severity of disease at the initiation of treatment.[51]

As an alternative to protein therapies, small-molecule drugs are attractive because they can be given orally, are more amenable to chemical optimization to improve pharmacokinetic properties, and have potential for broad tissue distribution, including delivery to the CNS.[12, 63] There are two small-molecule enzyme inhibitors approved to block the formation of storage materials: the SRTs miglustat (*N*-butyl-deoxynojirimycin, Zavesca) and eliglustat tartrate (Cerdelga) for Gaucher disease.[64, 65] Miglusat, an iminosugar, inhibits glucosylceramide synthase (GCS) to prevent production of the substrate for the defective enzyme GCase. It is also used for NPC and Tay–Sachs disease.[12, 66] However, the application of SRT to LSDs has been limited because of toxic side effects that make patient tolerability an issue.[67, 68] Eliglustat tartrate, a second generation GCS inhibitor, with a lower toxicity profile, was approved in 2014, but it is still unclear how widely it will be adopted for the clinical care of patients with type 1 Gaucher disease or other LSDs.[65] The overall approach of SRT could have other unfavorable pathological consequences in that inhibition of one enzyme to prevent the synthesis of precursor storage substrates might produce excessive storage of another macromolecule. For example, in NPC, acid lipase is responsible for the hydrolysis of cholesterol ester to free cholesterol, the disease storage material, and targeting this enzyme could reduce the buildup of cholesterol.[69] However, inhibition of acid lipase could cause accumulation of cholesterol esters in lysosomes, the phenotype of Wolman disease.

Another small-molecule target–based approach for the treatment of LSDs is the use of pharmacological chaperones (PCs) that specifically bind and stabilize mutant proteins to facilitate proper folding and trafficking to the lysosomes to increase activity levels of the enzyme in the lysosome.[63] Again, the advantages of this approach are that the molecules are low molecular weight, can be designed to cross the blood–brain barrier, and can be widely distributed to different cells and tissue types with no neutralizing antibodies generated. The

major class of PCs identified to date are enzyme inhibitors that mimic the natural substrates and bind to the active site, leading to stabilization and trafficking of the protein from the endoplasmic reticulum (ER) to the lysosome, where the lower acidic environment favors dissociation of the inhibitor from the protein and recovery of enzyme activity.[70] There are several PC series currently in preclinical and early clinical testing for Gaucher disease, Fabry disease, Morquio B disease, Krabbe disease, Pompe disease, the MPSs, Tay–Sachs disease, Sandhoff disease, and NPC.[63] For example, the chaperone ambroxol, which was found to interact with both the active and non-active sites of GCase, reportedly increased both GCase transport to lysosomes and enzymatic activity in skin fibroblasts derived from patients with type 1 and type 2 Gaucher disease.[71] It also reverted the inflammatory cytokine release from Gaucher disease macrophages derived from patient iPSCs.[72] An initial pilot clinical study in 12 patients for type 1 Gaucher disease showed individual improvements in disease-specific parameters measured, supporting further clinical testing.[73]

High-throughput screening (HTS) assays for the discovery of novel PCs have often used isolated recombinant enzymes, followed by chemistry optimization, to find a PC candidate in order to move forward into preclinical development.[70, 74] Although the use of recombinant enzymes has proven to be an important source for the discovery of inhibitory PCs, it is known that lysosomal enzymes are regulated by cellular metabolites, such as saponin for GCase, and therefore, screening using enzymatic preparations obtained from tissues or in a cell-based context have been used to identify compounds that are non-inhibitory pharmacological chaperones.[75] In a program to identify a PC for Gaucher disease, a screen using purified recombinant CGase first identified non-iminosugar small-molecule inhibitory chaperones.[74] Spleen homogenates from a Gaucher disease patient with the most common mutation, N370S, was used on a second screen that identified a new series of non-inhibitory chaperone molecule.[75] Non-inhibitory PCs are more appealing as chaperones because they can stabilize the active conformation of the enzyme by binding allosterically rather than in the active site. The use of a spleen enzyme preparation demonstrated a new screening paradigm using a disease-relevant mutant enzyme that functioned in the presence of natural modulators present in the spleen.

Primary LSD patient cells would provide a more disease-relevant model to screen for compounds that activate LSD enzymes, but this format has been difficult to implement for HTS because of the practical limitations related to generating the large number of cells needed. A few cell-based HTS assays to find small molecules that upregulate the activity of LSD enzymes have been reported for arylsulfatase A (ASA) in metachromatic leukodystrophy (MLD)[76] and β-galactocerebrosidase (GALC) in globoid cell leukodystrophy (GLD) or Krabbe disease,[77] using patient fibroblasts that were immortalized so that production of cells for HTS could be scaled up.

In vitro cell-based models

New technologies are enabling the development of *in vitro* cell-based disease models to study disease pathogenesis and to screen for new therapeutic molecules in relevant cell types, especially neuronal cells. Recent advances in iPSC technology offer great potential to generate new disease models for drug discovery.[25, 78, 79] Over the past 8 years, a rapidly

growing body of literature has demonstrated the use of iPSCs to derive tissue-specific cell types that are proving to be more predictive of the human condition than immortalized cell lines or primary rodent cultures. Many human cell types, such as neurons, cardiomyocytes, and hepatocytes, have been differentiated from iPSCs.[80] The iPSCs and differentiated mature cells generated from patient cells have been shown to exhibit the same genotypic, phenotypic, and functional characteristics of primary cells. Furthermore, the iPSCs and related progenitor cells can be renewed in culture to provide sufficient quantities of differentiated mature cells for phenotypic screening and evaluation of compound efficacy. Phenotypic screening with iPSC-derived patient cells can identify molecules that are relevant to disease pathogenesis, as well as disease phenotype, and casts a broader net to identify molecules that can regulate the underlying disease mechanisms described above. Unlike the molecular target–based drug discovery approach, a phenotypic screening approach looks for compounds relevant to disease pathogenesis and those molecules that modify a disease phenotype, for example, reducing storage macromolecules for LSDs, without necessarily needing to know a particular drug target. Primary hits identified in phenotypic screens may potentially act on different types of proteins (e.g., receptors, enzymes, transcription factors) and often implicate signaling pathways previously unknown to the disease.[81]

Human iPSC lines derived from patient primary cells have been generated for Fabry disease,[82] Gaucher disease,[83–87] GM1 gangliosidosis,[88] MPS I,[89] MPS IIIB,[90] MPSVII,[91] NPC,[92–95] Pompe disease,[96–99] and Batten disease (CNL2).[100, 101] The disease cellular phenotypes derived from the patient iPSCs are listed in Table 2. The efficiency in generation of iPSC colonies for Gaucher disease,[84] MPS IIIB,[90] Pompe disease,[97] and NPC[92] was reduced compared with that of wild-type cells. However, for NPC, it was found that addition of δ-tocopherol, a compound that inhibits the cholesterol accumulation phenotype in NPC fibroblasts, led to an efficient production of iPSC colonies.[42, 92] Recently, higher efficiency reprogramming vectors have become available, such as the Sendai virus vector, that have also greatly increased the yield of iPSC colonies,[102] which have been used to evaluate the treatment effects of enzyme replacement in Pompe disease, Gaucher disease, and Hurler syndrome. For Gaucher disease, iPSC macrophage cells have been shown to have a similar phenotype to patient monocyte-derived macrophages and have confirmed the efficacy of non-inhibitory chaperone molecules.[85] In iPSC-derived neuronal stem cells of NPC disease, nine compounds reported to have potential therapeutic effects were evaluated, but only three molecules—two cyclodextrin derivatives (2-hydroxypropyl-β-cyclodextrin (HP-β-CD) and methyl-β-cyclodextrin) and δ–tocopherol—were shown to reduce cholesterol storage.[92] HP-β-CD was also more potent in NPC1 neuronal cells compared to NPC1 fibroblasts, highlighting the existence of possible differential drug responses depending on the cell type and suggesting that these iPSC-derived cell models may allow the identification of compounds that specifically target neuronal pathophysiology.

In vivo animal models

Proof-of-concept studies of candidate therapeutic molecules for LSDs require rigorous testing in animal models with molecular pathophysiology and clinical phenotype as close to the human disease as possible in order to improve the predictability of drug efficacy in

human clinical trials. Genetically engineered mouse models are frequently used for *in vivo* preclinical efficacy studies, and many knockout or knockin models for LSDs have been reported over the last 20 years.[103] However, LSD mouse models have not always been successful in accurately reproducing the human disease phenotype. For example, the GCase gene (*Gba*) mutation N370S in GCase for Gaucher disease accounts for roughly 70% of the mutant alleles that are associated with the type I disease with relatively mild clinical manifestations in humans.[8] However, in a mouse *Gba* N370S knockin model, the mice die within 24 h of birth due to a skin defect similar to the knockout $Gba^{-/-}$ mice.[104] The mouse models with other *Gba* mutations, such as L444P, do not exhibit the same disease phenotypes either. These differences in disease phenotype seen in mouse models are likely to be a consequence of the species and/or genetic background differences.[105] Only a few LSD mouse models seem to reliably recapitulate the biological effects and symptoms observed in human LSDs. Even though the differences in size and metabolism of mice compared to humans are drawbacks, mouse models are still valuable and will continue to be used for initial studies because they are inexpensive, available in well-characterized inbred strains, and easy for introducing genetic modifications and producing large colonies of animals in a short time in order to test the effects of an adequate number of compounds *in vivo* for further selection of lead therapeutic development candidates.[106]

In a comprehensive overview by the European Medicines Agency (EMA) Committee for Orphan Medicinal Products (COMP) of mammalian models used in research for rare-disease research, 71% were in rats/mice, but 7% were in dogs, and 16% were in cats.[22] Large animal species, including cats, dogs, and pigs, offer several advantages in the translatability of preclinical studies to clinical trials that can complement and provide additional value to studies in mice. They are closer to humans in size and, in particular, brain size, which is advantageous for the study of LSDs with neurological disease symptoms. These species have more heterogeneity in genetic backgrounds, which better mimics human populations. They also have a longer life span to study the long-term effects of a therapy and are more amenable to procedures and manipulations, using instrumentation similar to that used in humans. Limitations of the use of large animal models include cost, difficulties in breeding large numbers of animals and maintaining colonies, increased amounts of test articles required, longer experimental times because of longer lifespans, and ethical considerations.

Currently, spontaneously occurring disease animal models have been described in dogs and cats for at least 18 LSDs, and 13 are available as colonies for research and preclinical testing.[107] Intravenous enzyme-replacement therapies have been studied in several LSD large animal models, including MPS I in dogs and cats[54, 108, 109] and MPS IV in cats.[110, 111] Studies in these LSD large animal models have provided critical preclinical data on safety and dose-related efficacy for IV delivery of ERTs, which was used to support their clinical development and approval.

For neurodegenerative LSDs, a drawback of the current ERTs administered by IV is that the proteins do not cross the blood–brain barrier and thus do not have an effect on the neurological phenotype. To bridge ERT therapy to neurodegenerative LSDs, clinical trials are underway to test direct administration into the CNS. Large animal models for disease in which the size and complexity of the brain are more similar to humans have allowed a

comparison of different CNS delivery methods for safety, distribution, and efficacy of potential therapeutics on neurological pathology that have been critical to answer preclinical questions and provide supportive data for testing in clinical trials with pediatric populations. In addition, these large animal models for disease can be useful in validating biomarkers and potential clinical outcome measures for the translation of therapies to humans. For example, a dog model was used in the development of an ERT for MPS IIIA (Sanfilippo syndrome), and ERT was delivered using various routes of CNS administration, including intrathecal (IT), intracisternal (IC), and intracerebroventricular (ICV), to test the long-term effects of the enzyme on brain pathology when administered to juvenile animals.[112] This study allowed the exploration of various dosing regimens for safety and efficacy, and the results showed that the primary storage of heparin sulfate (HS) and secondary storage of gangliosides were reduced when ERT was administered via all CNS routes, but there were differences in ERT penetration in some parts of the brain. The studies also explored the use of HS as a biomarker of disease status. In MPS I (Hurler syndrome), IT delivery using various dosing regimens was also tested in a MPS I dog model, and neurological responses to ERT therapy were measured using magnetic resonance imaging (MRI).[113] Neuroimaging in MPS I patients has shown that changes in white matter correlate with the cognitive impairment in the disease. In the MPS I dogs, it was found that measuring volumes of the white matter structure, the corpus collosum, with MRI may be a quantitative neuroimaging marker that could be translatable to human clinical trials as a measurable endpoint. A study of an ERT therapy of the mutated enzyme, tripeptidyl peptidase-1 (TPP1) in a dog model of late-infantile neuronal ceroid lipofuscinosis (CLN2 disease), administered directly to the CNS by IT delivery, strongly supported the initiation of IT administration of TPP1 in a clinical trial in children with CNL2 disease.[114] The CLN2 dog model closely mimics the human disease, and a therapeutic efficacy trial in dogs with this naturally occurring disease resulted in a dose-dependent attenuation of disease progression, improved neurological functions, and increased life span, in addition to the assessment of safety measures that decrease the risk/benefit ratio for the clinical trial in children. Another pivotal example of the use of large animal studies for the translation of a therapy into human clinical trials is the NPC disease cat model for the development of hydroxypropyl β-cyclodextrin (HPBCD), which is currently in phase 2 testing and examining IT administration to the CNS of juvenile NPC patients.[115] The cat NPC model shows neurological and biochemical abnormalities that closely mimic the juvenile form of the disease in humans, as determined in natural history studies of the disease in humans and in the cat model. A long-term study of IC CNS delivery of HPBCD in the cat model contributed critical preclinical information to the development program with respect to dose selection and route of administration.[116] More specifically, the cat model helped establish a well-defined pharmacokinetic/pharmacodynamic relationship in the CNS, toxicity (with the finding that HPBCD administration caused ototoxicity, which is now closely monitored in human clinical trials), and validated biochemical markers of disease severity and therapeutic effects that are specific to the neurological phenotype of the disease, such as cholesterol homeostatic responses upon redistribution of the main storage product of the disease, cholesterol, upon treatment with HPBCD.[117]

Conclusion

Highly predictive cellular and animal models are particularly critical for the clinical development of therapies for LSDs because of the small and mostly juvenile patient populations. Cellular models derived from patient iPSCs and larger animal models, such as cats, dogs, and pigs, are being used more frequently because of their closer pathogenesis and phenotype to the human disease. These models are becoming critical tools for the discovery of new targets and the understanding of common underlying cellular mechanisms that can lead to the discovery of treatments that restore common defective lysosomal functions and could be broadly applicable across diseases, particularly neurodegenerative diseases. Use of large-animal *in vivo* disease models have already had a significant effect in the translation of therapeutics for LSDs, both for those currently approved and in clinical testing. The predictability that both these *in vitro* and *in vivo* models have shown so far will make them critical for all stages in the discovery of new treatments for LSDs, from target identification, screening, lead optimization, proof-of-concept studies, and preclinical development.

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Table 1

List of lysosomal storage diseases

Disease name	Clinical name	Gene affected	Enzyme/protein deficiency	Primary storage materials
Aspartylglucosaminuria	AGU	*AGA*	Aspartylglucosaminidase	Glycoproteins
Cholesterol ester storage disease[a]	Wolman disease	*LIPA/LAL*	Acid lipase	Cholesteryl esters
Cystinosis[a]	Fanconi syndrome	*CTNS*	Cystine transporter	Cystine
Danon disease[a]	Danon disease	*LAMP2*	Lamp-2	Autophagic vacuoles[c]
Fabry disease[a]	Fabry disease	*GLA*	α-Galactosidase A	Globotriaosylceramide
Farber lipogranulomatosis[a]	Farber disease	*ASAH1*	Acid ceramidase	Ceramides
Fucosidosis[a]		*FUCA1*	α-L-fucosidase	Glycoproteins, glycolipids with fucose moieties
Galactosialidosis[a]		*CTSA*	Protective protein/cathepsin A β-galactosidase,[b] neuraminidase[b]	Glycolipids, mucopolysaccharides
Gaucher disease types I/II/III[a]	Gaucher disease	*GBA*	Glucocerebrosidase (β-glucosidase)	Glucocerebroside
Globoid cell leukodystrophy[a]	Krabbe disease	*GALC*	Galactocerebrosidase	Psychosine
Glycogen storage disease II[a]	Pompe disease	*GAA*	α-Glucosidase	Glycogen
GM1 gangliosidosis types[a] I/II/III		*GLB1*	β-Galactosidase	GM1 ganglioside
GM2 gangliosidosis type I[a]	Tay–Sachs disease	*HEXA*	β-Hexosaminidase A	GM2 ganglioside
GM2 gangliosidosis type II[a]	Sandhoff disease	*HEXB*	β-Hexosaminidase A & B	GM2 ganglioside
GM2 gangliosidosis[a]	AB variant	*GM2A*	GM2-activator deficiency	GM2 ganglioside
α-Mannosidosis types[a] I/II		*MAN2B1*	α-D-mannosidase	Mannose-containing oligosaccharides
β-Mannosidosis[a]		*MANBA*	β-D-mannosidase	Mannose-containing disaccharides
Metachromatic leukodystrophy[a]		*ARSA*	Arylsulfatase A	Sulfatides metachromatic leukodystrophy[a]
Metachromatic leukodystrophy[a]	Saposin B deficiency	*PSAP*	Saposin B	Sulfatides
Mucolipidosis type[a] I	Sialidosis types I/II	*NEU1*	Neuraminidase	Sialic acid–containing oligosaccharides
Mucolipidosis types[a] II/III	I-cell disease	*GNPTAB*	GlcNAc-1-phosphotransferase	Oligosaccharides, lipids, and glycosaminoglycans
Mucolipidosis type IIIC[a]	Pseudo-Hurler polydystrophy	*GNPTG*	Phosphotransferase γ-subunit	Same as above
Mucolipidosis type IV[a]		*MCOLN1*	Mucolipin1	Lipids, proteins
Mucopolysaccharidosis type IH[a]	Hurler syndrome	*IDUA*	α-L-iduronidase	Heparan sulfate, dermatan sulfate

Disease name	Clinical name	Gene affected	Enzyme/protein deficiency	Primary storage materials
Mucopolysaccharidosis type IS[a]	Scheie syndrome	*IDUA*	α-L-iduronidase	Heparan sulfate, dermatan sulfate
Mucopolysaccharidosis type II[a]	Hunter syndrome	*IDS*	Iduronate-2-sulfatase (12S)	Heparan sulfate, dermatan sulfate
Mucopolysaccharidosis type IIIA[a]	Sanfilippo syndrome	*SGSH*	Heparan-N-sulfatase	Glycosaminoglycans (GAGs)
Mucopolysaccharidosis type IIIB[a]	Sanfilippo syndrome	*NAGLU*	α-N-acetylglucosaminidase	Glycosaminoglycans
Mucopolysaccharidosis type IIIC[a]	Sanfilippo syndrome	*HGSNAT*	AcetylCoA:N-acetyltransferase	Glycosaminoglycans
Mucopolysaccharidosis type IIID[a]	Sanfilippo syndrome	*GNS*	N-acetylglucosamine 6-sulfatase	Glycosaminoglycans
Mucopolysaccharidosis type IVA[a]	Morquio syndrome	*GALNS*	N-acetylgalactosamine 6-sulfatase	Glycosaminoglycans, keratan sulfate
Mucopolysaccharidosis type IVB[a]	Morquio syndrome	*GLB1*	β-Galactosidase	GM1 ganglioside, keratan sulfate
Mucopolysaccharidosis type VI	Maroteaux–Lamy syndrome	*ARSB*	Arylsulfatase B	Glycosaminoglycans
Mucopolysaccharidosis type VII[a]	Sly syndrome	*GUSB*	β-Glucuronidase	GAGs: heparan sulfate, dermatan sulfate, chondroitin sulfate
Mucopolysaccharidosis type IX		*HYAL1*	Hyaluronidase-1	GAGs: hyaluronan
Multiple sulfatase deficiency[a]		*SUMF1*	Formylglycine-generating enzyme	Lipids, mucopolysaccharides
Neuronal ceroid lipofuscinosis, CLN1[a]	Batten disease	*PPT1*	Palmitoyl protein thioesterase	Lipofuscins (lipopigments)
Neuronal ceroid lipofuscinosis, CLN2[a]	Batten disease	*TPP1*	Tripeptidyl pepetidase 1	Lipofuscins
Neuronal ceroid lipofuscinosis, CLN3[a]	Vogt–Spielmeyer disease	*CLN3*	CLN3 (battenin)	Lipofuscins
Neuronal ceroid lipofuscinosis, CLN5[a]	Batten disease	*CLN5*	CLN5	Lipofuscins
Neuronal ceroid lipofuscinosis, CLN6[a]	Batten disease, late infantile	*CLN6*	CLN6 (linclin)	Lipofuscins
Neuronal ceroid lipofuscinosis, CLN8[a]	Northern epilepsy	*CLN8*	CLN8	Lipofuscins
Niemann–Pick disease types A/B[a]	Niemann–Pick disease	*SMPD1*	Acid sphingomyelinase	Sphingomyelin
Niemann–Pick disease type C1[a]	Niemann–Pick disease	*NPC1*	NPC1 protein	Cholesterol
Niemann–Pick disease type C2[a]	Niemann–Pick disease	*NPC2*	NPC2 protein	Cholesterol
Pycnodysostosis		*CTSK*	Cathepsin K	Collagen
Schindler disease types[a] I / II	Schindler disease	*NAGA*	α-N-acetylgalactosaminidase	Glycoproteins, glycolipids
Sialic acid storage disease[a]	Sialuria, Salla disease	*SLC17A5*	Sialin (sialic acid transporter)	Sialic acid

Note: The list of LSDs was obtained from the website for the Lysosomal Disease Network (http://www.lysosomaldiseasenetwork.org). The information on affected genes, protein deficiency, and storage materials was obtained from the Genetics Home Reference website (http://ghr.nlm.nih.gov).

[a]Neuronal involvement was reported.

[b] Secondary deficiency.

[c] Autophagic vacuoles are located in cytosol.

Table 2

Disease phenotypes identified in the iPSC lines derived from LSD patient cells

Disease	Original cell (mutation)[a]	Differentiated cells	Disease phenotype described	References
Fabry disease	GM00107 (Trp162Ter)	iPSCs	Gb3 accumulation	82
Gaucher disease				
	(N370S/N370S; L444P/RecNciI; L444P/L444P)	Macrophages	Sphingolipids accumulation	83
	(L444P/G202R)	Neurons/macrophages	Sphingolipids accumulation	84
	(N370S/N370S; N370S/c.84dupG; IVS2 +1G > A/L444P)	Macrophages	Glucocerebroside and glucosylsphingosine	85
	D(N370S/N370S; L444P/L444P)	Dopaminergic neurons	Glucosylceramide and a-synuclein, autophagic and lysosomal defects	86
	(N370S/N370S; W184R/D409H, L444P/RecNci1; L444P/L444P)	Hematopoietic progenitors and aberrant myelopoiesis	Decreased erythroid potential	87
GM1 gangliosidosis	GM02439, GM05652	Neural progenitor	GM1 ganglioside accumulation	88
Mucopolysaccharidosis I	(Y167X/W402X; W402X/W402X)	Hematopoietic stem cells	Glycosaminoglycan accumulation	89
Mucopolysaccharidosis IIIB	((c.531+1G>C); R482W/R482W)	Neural stem cells/neurons	Glycosaminoglycan accumulation	90
Mucopolysaccharidosis VII	GM02784	Neuron stem cells		91
Neuronal ceroid lipofuscinosis, CLN2	(*IVS5/E6*)	Neural progenitor cells, neurons	Increased lipid droplets, increased vacuoles	101
Neuronal ceroid lipofuscinosis, CLN3	(Δex7/8/Δ7/8; IVS13/E15)	Neural progenitor cells, neurons	Increased LAMP-1 staining, increased vacuoles	101
Niemann–Pick C				
	(I1061T/P237S)	Neural stem cells/neurons	Cholesterol accumulation	92
	(1628delC/E612D)	Neurons	Cholesterol accumulation	95
	(I1061T/I1061T)	Neural stem cells/neurons	WNT signaling pathway abnormality	93
	GM18453 (I1061T); GM03123(P237S/I1061T); GM22870 ((1920 delG)/1009G > A); GM22871 ((1920 delG)/1009G > A)	Hepatocytes/neurons	Autophagic flux defect	94
Pompe disease				
	(D645E/D645E; D645E/D645E; c.1935C.A/c.2040 +1G.T; Y354X/D645E)	Cardiomyocytes	Glycogen accumulation, low GAA activity	97
	(c.796 C>A and c.1316 T>A)	Cardiomyocytes	Glycogen accumulation, low GAA activity	99
	GM20124; GM11661	iPSCs	Glycogen accumulation	96
	GM20089 (Δex18/Δex18); GM04912 (1441delT/2237G3A)	Cardiomyocytes	Golgi-based glycosylation deficit	98

[a]The GM number indicates that the patient cell line was obtained from Coriell Cell Repositories and mutations are listed on their website (https://catalog.coriell.org/), but in some cases, the mutations were not reported.