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Lysosomal storage disorders with neurological manifestations

Vikas Munjal[1], Maria T. Clarke[2], Joshua Vignolles-Jeong[1], Jasmine A. Valencia[2], Meika Travis[1], Lluis Samaranch[1]

[1]Department of Neurological Surgery, The Ohio State University, 410 W 10th Ave, Columbus, OH 43210, USA.
[2]Department of Surgery, University of California San Francisco, 400 Parnassus Ave, San Francisco, CA 94103, USA.

Correspondence to: Prof. Lluis Samaranch, Department of Neurological Surgery, The Ohio State University, 410 W 10th Ave, Columbus, OH 43210, USA. E-mail: lluis.samaranch@osumc.edu

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Abstract

Lysosomal storage disorders (LSDs) constitute a large group of rare, multisystemic, progressive, inherited disorders of metabolism. The aberrant metabolic processes often lead to the cellular accumulation of incompletely metabolized macromolecules or their metabolic byproducts. Most of the patients affected by LSD can experience a variety of neurological presentations including, but not limited to, psychiatric complications, seizures, and/or developmental delays. The onset of symptoms can range from birth to adulthood, and disease severity can vary. Since there is significant overlap in the symptomatology of LSDs, diagnosis is typically confirmed through biochemical and molecular assays. There are currently no approved cures for any LSDs; however, in most cases, treatment of symptoms can lead to better outcomes and improvements in quality of life. The use of hematopoietic stem cell transplantation, enzyme replacement or substrate reduction therapy, and viral vector gene transfer is the subject of many ongoing and completed clinical trials. In this mini review, we provide an overview of LSDs with neurological manifestations, describe the current endeavors in alleviating peripheral symptoms and discuss effective therapeutics strategies.

Keywords: Lysosomal storage disorders, hematopoietic stem cell transplantation, enzyme replacement therapy, gene therapy, AAV viral vectors

OVERVIEW OF LYSOSOMAL STORAGE DISORDERS

Lysosomal storage disorders (LSD) are characterized by the accumulation of toxic substrates in the cells due to mutations in genes encoding lysosomal proteins[1]. There are over seventy distinct disorders classified as LSDs, many of them having an affected lysosomal hydrolase enzyme which is directly implicated in the aberrant processing and degradation of the accumulating substrates[1,2]. Others are due to mutations on lysosomal membrane proteins or proteins involved in the transport of lysosomal enzymes[1]. In addition to the specific mutation involved, oxidative stress and inflammation are also key in LSD pathophysiology and define its progression. Autoimmune responses to specific accumulated macromolecules or metabolites, as well as neuroinflammation-driven microglial activation and astrogliosis, cause activation of apoptotic mechanisms leading to irreversible cellular damage and organ dysfunction[3,4].

Most LSDs are monogenic, autosomal recessive disorders, although three of them (Fabry, Danon, and Hunter syndromes) are X-linked recessive. A study of the Australian population estimated that collectively, LSDs have a combined prevalence of 1 per 7700 live births, but individually are rare-to-ultra-rare; Gaucher disease is the most common LSD, with a prevalence of 1 per 57,000 while sialidosis has the lowest estimated prevalence with 1 in 4.2 million[5]. Despite the global distribution of LSDs, some syndromes cluster in high-risk populations. For example, the Ashkenazi Jewish population has a higher prevalence of Gaucher, Tay-Sachs, and Niemann-Pick diseases[6], Scandinavian and Russian populations have an increased frequency of Hurler syndrome, and Finnish populations have a higher incidence of aspartylglucosaminuria or neuronal ceroid lipofuscinosis[7,8]. However, these prevalence statistics most likely represent an underestimation of their true magnitude due to the frequent misdiagnosis or underdiagnosis that occurs in LSDs[9]. This, in combination with the clinical heterogenicity even within the same disorder, is a significant complication for epidemiological studies in LSD, leading to poor or incomplete data that do not reflect the real-world impact of these diseases in the community.

A few LSDs do have treatment options, including both enzyme replacement therapies (ERTs), which can alleviate some symptoms by providing the missing enzyme[10-12], and substrate reduction therapy (SRT), which can reduce the amount of accumulated toxic substrate by decreasing its biosynthesis[13]. Although ERT is often a viable option for the treatment of peripheral organs of non-neurological LSDs, it is not an option for LSDs with neurological involvement because of the inability of most of the enzyhmes to cross the blood-brain-barrier (BBB). Cerebrospinal fluid (CSF) delivery of the enzymes has emerged as a solution to circumvent the BBB[14], and several clinical trials have been initiated to test safety and efficacy. Current data show some degree of effect in MSP and CLN clinical trials, although efficacy is limited if not treated in very early stages. An alternative to rescue or restore the loss-of-function in LSDs with neurological involvement is gene therapy. Adeno-associated viruses (AAVs) are the most common vector used to direct the expression of the therapeutic transgene into the central nervous system (CNS)[15], and several clinical trials are in progress to probe the safety and efficacy of rescuing the enzymatic activity[16]. In addition to bypassing the BBB, one of the advantages of the CNS delivery of viral vectors is axonal transport, the capacity of the viral particles to be transported from the injection site to distal interconnected structures[17-19]. Axonal transport facilitates broader distribution of the therapeutic into cortical and subcortical structures of brain compared to ERT injections into the CSF of brain parenchyma[20].

CLINICAL FEATURES AND NEUROLOGICAL MANIFESTATIONS

The inability of lysosomes to perform their physiological function because of an enzyme deficiency is the common feature of all LSDs. There is a great degree of variability in age of onset and clinical manifestations, depending on the accumulating substrate involved, which complicates efforts to standardize the clinical features [Table 1]. Common clinical features include physical development delay, difficulty of breathing,

Table 1. A collection of neuropathic lysosomal storage disorders

Disease	Defective protein	Neurological clinical signs	Gene (locus)	OMIM
Aspartylglucosaminuria	Aspartylglucosaminidase	ES, DD	*AGA* (4q34.3)	208400
Fabry disease	Alpha-galactosidase	CC, CAT, LK, DF, CV, DP, PN	*GLA* (Xq22.1)	301500
Farber disease	Acid ceramidase	DD	*ASAH1* (8p22)	228000
Fucosidosis	α-L-fucosidase	MS, ES, DD	*FUCA1* (1p36.11)	230000
Galactosialidosis	Protective protein cathepsin A	OA, MS, DF, AX, DD	*CTSA* (20q13.12)	256540
Gaucher disease Type II	Glucocerebrosidase	MS, DF, DD	*GBA* (1q22)	230900 231000
Gaucher disease Types III	Glucocerebrosidase	OA, MS, AX, ES, DP, DD	*GBA* (1q22)	230900 231000
Glycogenosis II/Pompe disease	α-Glucosidase	DD	*GAA* (17q25.3)	232300
GM1 gangliosidosis (Types I, II, III)	GM1-β-galactosidase	OA, CA, AX, ES, DP, DD	*GLB1* (3p22.3)	230500 230600 230650
GM2 gangliosidosis, AB variant	GM2 activator protein	MS, CA, AX, ES, DP	*GM2A* (5q33.1)	272750
GM2 gangliosidosis, Sandhoff variant	β-Hexoaminidase A + B	OA, MS, MC, CA, AX, ES, DP	*HEXB* (5q13.3)	268800
GM2 gangliosidosis, Tay-Sachs variant	β-Hexosaminidase A	OA, MS, MC, CA, AX, ES, DP, DD	*HEXA* (15q23)	272800
Krabbe disease	Galactocerebrosidase	LK, MC, PN, AX, ES, DP, DD	*GALC* (14q31.3)	245200
Metachromatic leukodystrophy	Arylsulfatase A	LK, DF, PN, CA, AX, DP, DD	*ARSA* (22q13.33)	250100
MPS I (Hurler, Scheie, Hurler-Scheie)	α-L-iduronidase	CC, DF, DD	*IDUA* (4p16.3)	607014 607015 607016
MPS II (Hunter)	Iduronate-2-sulfatase	DP, DD, DF, OA, MS, MC	*IDS* (Xq28)	309900
MPS III A (Sanfilippo)	heparan N-sulfatase	DP, DD, DF, OA, MS, MC	*SGSH* (17q25.3)	252900
MPS III B	α-*N*-Ac-glucosaminidase	DP, DD, DF, OA, MS, MC	*NAGLU* (17q21.2)	252920
MPS III C	heparan-α-glucosaminide N-acetyltransferase	DP, DD, DF, OA, MS, MC	*HGSNAT* (8p11.21-p11.1)	252930
MPS III D	N-acetylglucosamine 6-sulfatase	DP, DD, DF, OA, MS, MC	*GNS* (12q14.3)	252940
MPS IV A (Morquio A)	N-acetylgalactosamine 6-sulfatase	OA, CC, DF, DD	*GALNS* (16q24.3)	253000
MPS IV B	Beta-galactosidase	OA, CC, DD	*GLB1* (3p22.3)	253010
MPS VI (Maroteaux-Lamy)	Arylsulfatase B	CC, DD	*ARSB* (5q14.1)	253200
MPS VII (Sly)	Beta-glucuronidase	OA, DD	*GUSB* (7q11.21)	253220
Mucolipidosis II	UDP-N-Ac-glucosaminyl phosphotransferase	OA, CC, DF, CA, DD	*GNPTAB* (12q23.2)	252500
Mucolipidosis IV	Mucolipin 1	CC, AX, DD	*MCOLN1* (19p13.2)	252650
Multiple sulfatase deficiency	Sulfatase modifier protein	PN, DD	*SUMF1* (3p26.1)	272200
Neuronal Ceroid Lipofuscinosis 1	Palmitoyl-protein thioesterase 1	OA, RP, MS, CA, AX, DP, DD	*PPT1* (1p34.2)	256730
Neuronal Ceroid Lipofuscinosis 2	Tripeptidyl peptidase 1	OA, RP, MS, CA, AX, DP, DD	*TPP1* (11p15.4)	204500
Niemann-Pick type A (Neurovisceral Type)	Sphingomyelinase	OA, DD	*SMPD1* (11p15.4)	257200
Niemann-Pick type B (Visceral Type)	Sphingomyelinase	OA, DD	*SMPD1 (11P15.4)*	607616
Niemann-Pick type C1	NPC1	OA, MS, AX, ES, DP, DD	*NPC1* (18q11.2)	257220
Niemann-Pick type C2	NPC2	OA, MS, AX, ES, DP, DD	*NPC1* (18q11.2)	607625
Saposin defect, Gaucher type	Saposin C	OA, MS, AX, ES, DP, DD	*PSAP* (10q22.1)	610539
Saposin defect, generalized type	Prosaposin	AX, CA, DD	*PSAP* (10q22.1)	611721
Saposin defect, MLD type	Saposin B	LK, DF, PN, CA, AX, DP, DD	*PSAP* (10q22.1)	249900

Schindler disease	α-N-acetylgalactosaminidase	OA, MS, ES, DD	*NAGA* (22q13.2)	609241
Sialic acid storage disease, infantile (ISSD), adult (Salla)	Sialin	OA (ISSD), AX (Salla), DD	*SLC17A5* (6q13)	604369
Sialidosis	Sialidase	OA, CAT, MS, AX, ES, DD	*NEU1* (6p21.33)	256550
Wolman disease/CESD	Acid lipase	AX, DP, DD	*LIPA* (10q23.31)	278000
α-Mannosidosis	α-Mannosidase	CC, CAT, DF, ES, DD	*MAN2B1* (19p13.13)	248500
β-Mannosidosis	β-Mannosidase	DF, ES, DD	*MANBA* (4q24)	248510

OA: Optic Atrophy; RP: retinitis pigmentosa; CC: corneal clouding; CAT: cataracts; LK: leukodystrophy; MS: myoclonic seizures; DF: deafness; MC: macrocephaly; PN: peripheral neuropathy; CA: cortical atrophy; CV: cerebrovascular events; DD: developmental delay; DP: dementia psychosis.

"cherry-red spot", deafness, bone deformities, hydrops, seizures, and ataxia[21]. The onset of symptoms varies greatly, from birth to late adolescence and adulthood[21]. Many patients with LSDs are born with no apparent pathological phenotype for several months after birth, until hepatosplenomegaly or cardiomyopathy is evident, generally in non-neuropathic types[21]. Types with predominant neuropathic involvement are often associated with cognitive and motor developmental delay[22], leading to mental retardation, progressive neurodegeneration, and premature death[23]. Even then, these distinctions are not always clear. Gaucher's disease, for example, exemplifies the wide array of clinical manifestations associated with LSDs. Type I patients have no neurological symptoms, while type II and III patients can experience ataxia, myoclonic seizures, deafness, dementia, and psychosis[21,24]. This variability in clinical features is related to the particular defect of the malfunctioning enzyme and the accumulating substrate. In the case of Gaucher, glucosylceramides (GCS) accumulate due to deficiency in glucocerebrosidase activity[25], and different origins of GCS cause different clinical manifestations and disease progression. In the brain, GCS are derived primarily from gangliosides, while in peripheral organs, GCS are derived from the breakdown of blood cells. Type I maintains sufficient residual enzyme activity in the brain to avoid ganglioside GCS accumulation, while types II and III patients show lower residual enzymatic activity in the brain and GCS accumulation inside the neurons leading to aberrant inflammatory and apoptotic responses[24]. GM1 and GM2 gangliosidosis and metachromatic leukodystrophy (MLD) are typically associated with more severe neurologic outcomes, including visual loss, cerebellar atrophy associated with ataxia, seizures, and peripheral neuropathy[21]. Type A and Type B Niemann-Pick disease (NPD-A and NPD-B, respectively) are another notable example of a pair of LSDs that share a common deficient enzyme, acid sphingomyelinase (ASM), which is crucial in sphingolipid homeostasis and membrane turnover, but presents with different symptomatology[26]. Individuals with both NPD-A and NPD-B exhibit hepatosplenomegaly, progressive lung disease, and failure to gain weight[27,28], and develop large, lipid-laden foam cells accumulate in affected organs as a result of sphingolipid backup[29]. Involvement of the central nervous system in enzyme deficiency is the primary distinguishing feature between NPD-A and NPD-B; NPD-A is also characterized by rapidly progressive neurodegeneration. Most infants with NPD-A are diagnosed early in their first year of life, do not meet critical developmental milestones, and typically do not survive past their third year of life[27,30]. In stark contrast, patients with NPD-B display no signs of central nervous system involvement and often live into adulthood with medical management of peripheral symptoms. Small deletions or nonsense mutations in the ASM polypeptide, as well as missense mutations in the gene encoding the production of the acid sphingomyelinase, cause Type A. In Type B, a separate missense mutation produces defective acid sphingomyelinase with minimal residual activity, leading to the nonneuronopathic presentation of the disease[31]. While results from clinical trials have demonstrated that ERT can address the peripheral pathology of NPD[32,33], potential gene therapy treatments to address the CNS pathology seen in NPD-A are in the pre-clinical stages[34].

Age-of-onset of the disease phenotype also has a significant impact on both severity and progression of the disease, generally led by the genomic background or the causal mutations involved in the disease. Fabry disease, for example, is an X-linked LSD caused by deficiency of the enzyme alpha-galactosidase, which results in the accumulation of globotriaosylceramide in the brain, liver, skin, heart, and kidneys[35]. In early-onset Fabry disease, patients typically show hallmark symptoms including angiokeratoma, hypohidrosis, corneal opacities, and neuropathic pain, often in the form of acroparesthesias[35,36], while late-onset patients may instead present severe renal, cardiac, and vascular manifestations as the disease progresses[37]. Especially in young/early-onset patients, LSDs are also often misdiagnosed as psychiatric disorders due to the presence of similar symptoms such as anxiety, restlessness, aggression, and increased sensitivity to touch[38]. As the disease progresses, other psychiatric symptoms start to be more evident, like hallucinations, psychosis, and dementia[38]. In patients with late-onset Tay Sachs Disease, up to 20%-40% of affected individuals will present with psychiatric symptoms years before the onset of motor deficits[39]. Infantile diagnoses of MLD present with motor symptoms such as gait instability and loss of motor developmental milestones, while adults with MLD demonstrate behavioral disturbances and dementia, which may be mistakenly diagnosed as psychosis[40]. Patients with Niemann-Pick Type C (NP-C) may develop neuropsychiatric symptoms at any point in their disease progression, including agitations, sleep disorders, and depression[41].

Despite advances in the identification and treatment of LSDs, affected individuals are often diagnosed with a psychiatric disorder without appropriate follow-up for their underlying disease. One study reported an average delay of 6.2 years between initial symptom presentation and diagnosis in patients with NP-C, as the majority of patients who presented with psychosis as their initial symptom of disease did not demonstrate any neurological exam abnormalities and were given a diagnosis of schizophrenia or other forms of psychosis[42]. Individuals who are being evaluated for a psychiatric disorder, particularly those who also present with motor deficits or who do not respond to standard psychiatric management, may greatly benefit from a rigorous metabolic work-up[38].

DIAGNOSIS, GENETIC TESTING AND METABOLOMICS

Antecedents of the disease in the family (proband), or positive screening of family members (carrier) can increase the identification of presymptomatic individuals through prenatal testing or newborn screening, but most often, the lack of symptoms, especially in the carriers, causes diagnostic confirmation to occur after multiple organs have been irreversibly damaged and therefore therapeutic interventions are less efficacious[43]. Early identification of positive cases at prodromal stages would facilitate the design and execution of a clinical management plan when the therapeutic intervention has a better chance of success[44]. However, the ethical dimension of early-in-life screening programs is still an active discussion among genetic counselors, health-care professionals, and health policymakers[45].

In general, the onset of symptoms may present anywhere between infancy and later adulthood and continue worsening over time, chronifying the disease or causing death[21]. Although mutational and enzymatic analyses stablish the diagnostic in all cases, biochemical markers in presymptomatic individuals can predict cases in some LSD. For example, the mucopolysaccharidoses group of LSDs, increased urinary excretion of glycosaminoglycans can be detected, while increased levels of oligosaccharides will be detected in the urine of patients in the oligosaccharidoses group[46]. In Pompe Disease, increased blood levels of creatine kinase can be found, while patients with Gaucher or Niemann-Pick C Disease may demonstrate elevated levels of chitotriosidase in serum[46]. Currently, measurement of lysosomal enzyme activity can reliably diagnose most LSDs by identifying a severe deficiency. Fluorometry[47,48], tandem mass spectrometry[49,50], and radioactive assays are the principal techniques for enzymatic activity assessment. However, these assays can be tissue-dependent, leading to false-negative results if the wrong specimen is used in the assay[51]. Also, it is

important to know that the complete absence of lysosomal enzyme activity generally leads to an LSD diagnosis, but conversely, normal lysosomal enzyme activity is not enough to discard LSD diagnosis if there is a clinical phenotype suggestive of LSD[46]. In certain variant forms of GM2 gangliosidosis, Krabbe disease, MLD, and Gaucher disease, enzyme levels could be normal, but disease phenotype may surface due to a defect in saposins, a group of glycoproteins that activate lysosomal hydrolases related to sphingolipid metabolism[52].

Genetic confirmatory analysis is being utilized with increasing frequency as laboratory techniques and bioinformatic data management are advancing. Conventional methods for genetic confirmation of LSDs like MLPA, RFPL, or Sanger sequencing are limited by the fact that they analyze a single mutation at a time. Modern techniques like next-generation sequencing (NGS), including whole-genome sequencing and whole-exome sequencing, can identify causal genetic variants of the monogenic disease, including neurometabolic disease streamlining the mutation screening in the diagnostic process[53]. These high-throughput DNA sequencing technologies are time and cost-effective and can interrogate a large number of genes in a single reaction[54,55]. For example, in 2013, Hoffman and colleagues concluded that NGS can be superior in identifying Tay-Sachs Disease carriers compared to traditional enzyme and genotype analyses which can be limited by both false positives and negatives[56], and in 2016, Yoshida and colleagues demonstrated the application of NGS in prenatal diagnosis of Gaucher disease even in the absence of prior genetic information from the family[57].

New challenges have been introduced as these high-throughput technologies advance. Higher sophistication of laboratory techniques, including new reagents and new equipment, is needed, but the biggest challenge lies at the bioinformatic level, which includes a new requirement for 'big data' management and accessible data interpretation[58]. Certainly, new intersections between different specialties like informatics, statistics, and biology, as well as new emerging fields of science like data mining or artificial intelligence, are facilitating the accessibility of these new techniques to the clinic. Genetic counseling is also an important consideration of these new techniques, especially in LSDs[59]. Counseling for families with LSD generally include education of the disease, inheritance patterns, recurrence risks, and implications of the diagnosis, progression, and needs for ongoing medical and family management. Currently, no curative therapies are approved, with current disease management primarily focusing on the control of symptoms in LSD patients via ERT. Counseling and carrier screening are essential in the management and mitigation of the incidence and prevalence of theses genetic disorders. One successful example of management through genetic counseling is in the Ashkenazi Jewish population[60], where carrier screening in individuals with Ashkenazi ancestry resulted in a dramatic decrease in the incidence of some LSD, including Tay Sachs, Canavan, Gaucher, or Niemann-Pick[61,62].

AVAILABLE TREATMENT AND CLINICAL TRIALS FOR LSD WITH CNS INVOLVEMENT

Several symptomatic treatment options are available for LSDs. Enzymatic or protein deficiencies can be restored by cellular or recombinant proteins like hematopoietic stem cells transplantation (HSCT) or ERT, respectively, and the buildup of metabolites can be reduced through SRT. Biological treatments can be combined with physical and occupational therapy to improve outcomes[63].

Hematopoietic stem cells transplantation

In use for over 20 years, HSCT originally involved bone marrow transplantation and has advanced to using unrelated umbilical cord blood as a source of stem cells[64-66]. HSCT is primarily performed in patients with mucopolysaccharidoses and leukodystrophies and ultimately functions as an ERT. Healthy engrafted donor cells not only provide a continuous endogenous supply of enzyme in the extracellular space and into the

blood circulation, but also microglia derived from the donor stem cells can migrate to the brain and secrete functional enzyme in the CNS improving neurocognitive function and quality of life[67]. HSCT is the gold standard treatment in young Hurler's syndrome patients with mild to no cognitive impairment, showing a significant reduction in mortality[65,68,69]. Important limitations are associated with this technique, including graft failure and high morbidity and mortality rates. Leukocyte enzyme levels may also not reach target levels for several years, potentially limiting the effectiveness of this treatment modality in patients with rapidly progressing disease. Additionally, in treated Hurler's syndrome patients, skeletal complications continue to develop, and the majority of the patient will require surgical intervention[65,66,70].

Enzyme replacement therapy

ERT has been shown to be an effective therapy in ameliorating the visceral, hematological, and biochemical manifestations for some LSDs, substantially improving the patient quality of life. Treatments for Gaucher, Fabry, MPS, and Pompe diseases are a clear example of successful ERT. Weekly or bimonthly intravenous injections provide broad biodistribution of the recombinant enzyme that is internalized by cells and conducted to the lysosomal compartment. This internalization is mediated by mannose or the mannose-6-phosphate receptor following the endocytic route and rescuing the lysosomal enzyme deficit. Despite the successes of ERT, the production of IgG antibodies against recombinant enzyme (anti-drug antibodies) can lead to diminished activity or cellular uptake[71]. However, recent studies reported the benefit of the immune tolerance induction in some patients[72]. This technique aims to diminish the development or minimize the consequences of the response against the ERT, especially in cross-reactive immunologic material -negative or null patients[73,74]. Another major limitation is the inability of intravenously administered functional enzyme to cross the BBB. This is especially problematic given that about two-thirds of LSDs present with neurologic symptoms[75]. Current research is focused on ensuring the accessibility of the CNS to treatment modalities, including modifications to the enzyme that allow passage through the BBB, delivering a high intravenous dose of enzyme, or intrathecal injections[75].

Substrate reduction therapy

SRT aims to mitigate the effects of substrate accumulation. SRT uses small molecules to inhibit the biosynthesis of compounds that are accumulating problematic levels in the absence of functional lysosomal enzyme[76,77]. In Gaucher disease, accumulation of glucosylceramide can be targeted with miglustat, an oral glucosylceramide synthase inhibitor. This decreased rate of accumulation allows residual enzyme activity to more effectively clear lysosomal stores of glucosylceramide, slowing disease progression[78]. Miglustat has also demonstrated similar efficacy in the treatment of NPD-C, with the potential for extending patients' lives[79]. More recently, eliglustat, a more potent ceramide-mimetic inhibitor that demonstrated less severe side effects than miglustat, was developed. Unfortunately, eliglustat does not cross the BBB, limiting its efficacy in treating the neuropathic types of Gaucher disease[80,81]. SRT and ERT can be used in conjunction with other therapies, including each other. In Fabry disease mouse models, the therapeutic effect of the combination of SRT and ERT was found to be additive and complementary[82]. The combination treatment may allow for reduced frequency of ERT, potentially leading to an increase in quality of life due to decreased dependency on enzyme infusions[83]. This combination of treatments could be beneficial for patients who demonstrate a complete absence of enzymatic activity.

Gene therapy

Gene therapy has seen substantial improvement in recent years, especially for LSDs. With this approach, functional copies of defective genes are introduced into the human cells to treat or prevent diseases. This "genome editing" technique can be performed *ex-vivo* or *in-vivo*. Adeno-associated viral vectors (AAV) and retroviral vectors have been extensively used in pre-clinical studies of LSDs with CNS implications like mucopolysaccharidosis types I[84], III[85], and VII[86], Krabbe disease[87], metachromatic leukodystrophy[88],

Niemann-Pick[34], ceroid lipofuscinosis[89], or GM1 gangliosidosis[90]. One major benefit of gene therapy over ERT is that gene therapy is administered in a single dose. During transfection, the new genetic material is introduced into the cell, engaging existing cellular machinery to produce the protein of intereste, with the goal of restoring missing functionality. These vectors can be introduced intravenously, although, for neuropathic LSD, parenchymal or intraventricular/intrathecal have been demonstrated to have better results[16,91,92]. As with other systemic approaches, the ability to cross the BBB is also an important limitation for vector distribution into the brain. Since AAV serotype 9 is the only vector capable of crossing the BBB, it is currently the only viable option for AAV-based systemically administered therapy[93,94]. To achieve significant and widespread effect in the CNS after systemic delivery, large volumes of the viral vector are required, and these high doses have been associated with sensory neuron toxicity in some cases[95,96]. In contrast, CSF delivery needs less total load of viral vector to achieve significant levels of transduction in the brain and spinal cord[94,97], but recently, dorsal root ganglia histopathology with no clinical signs has also been reported in a meta-analysis in non-human primates[98]. Parenchymal delivery, although technically more invasive, has so far shown no adverse effects related to transgene-overexpression and remains the platform of choice in multiple clinical trials. Once in the brain, there are two characteristics differentiate gene therapy from other therapeutic approaches: axonal transport and the bystander effect. Axonal transport is the property by which viral particles use the projections between neurons to travel to different brain structures, increasing the distribution beyond the injection site[17,99]. Convection enhanced delivery is a bulk-flow mechanism used to convey and distribute macromolecules into brain parenchymal tissue improving the local distribution into the injection site and reducing the off-target delivery[100,101]. Altogether, this efficient local transduction improves the distal transduction by reaching a larger number of projections. Because most affected enzymes in CNS-involved LSDs are secreted, treatments benefit from the cross-correction of bystander cells. Once some of the cells are transduced by the vectors, functional enzymes are produced then secreted and internalized by mannose-6-phosphate receptors-mediated endocytosis by other neighboring enzyme-deficient cells[102], enhancing the distribution of the therapeutic effect into cells that were not transduced[34,103]. Multiple examples define the proof-of-concept for this therapeutic approach in LSDs,[104-106] but without doubt, from the early mouse models for Mucopolysaccharidosis[107] to the current large animal models for LSDs[108-110], the existence of a large selection of animal models propelled the better understanding of the underlying disease mechanisms, accelerating the pre-clinical development[111]. However, species-specific differences in cellular metabolism are the basis of differences in the pathological phenotype that sometimes do not effectively recapitulate what is seen in patients, especially in CNS, where brain complexity can be challenging to model in animals.

Because of the poor efficacy in the CNS of the available therapies, including systemic ERT, most of the current clinical trials involve the use of gene therapy to correct neurological defects. Detailed information of the clinical trials is available through *clincialtrials.gov*, and the most recent active trials for lysosomal storage disorders with neurological deficits are summarized in Table 2.

ONGOING DEVELOPMENT: NEW APPROACHES, NEW HOPES

A wide collection of studies continues to examine the utility of AAV and its related variants in the treatment of neuropathic LSD. New natural capsids are being discovered by high-throughput screening or isolated from other vertebrate species that are under investigation for potential therapeutic use. Novel capsids are also being engineered by rational design or directed evolution. One notable example is the discovery of AAV-PHP.B, an engineered capsid identified by Cre recombination-based AAV targeted evolution, a directed evolution method that uses Cre/lox technology[112], and is capable of crossing the BBB and transducing the CNS[113]. More recently, barcoded rational AAV vector evolution optimized a screening process used to identify the optimal capsid from multiple rounds to a single *in vivo* screening round[114].

Table 2. Active clinical trials for neuropathic lysosomal storage disorders

Disease	Vector	Phase	NCT Number	Status
Fabry	AAV	Phase 1/2	NCT04455230	Recruiting
Fabry	AAV2/6	Phase 1/2	NCT04046224	Recruiting
Fabry	AAV 4D-310	Phase 1/2	NCT04519749	Recruiting
Fabry	Lentiviral-HSC GT	Phase 1/2	NCT03454893	Recruiting
Fabry	Lentiviral-HSC GT	Phase 1	NCT02800070	Active, not recruiting
GM1 Gangliosidosis	AAVrh.10	Phase1/2	NCT04273269	Recruiting
GM1 Gangliosidosis	AAVHu68	Phase 1/2	NCT04713475	Recruiting
GM1 Gangliosidosis Type II	Aav9	Phase 1/2	NCT03952637	Recruiting
GM2 Gangliosidosis (Inftantile-Onset)	AAV9	Phase 1/2	NCT04798235	Recruiting
Krabbe	HSC GT + AAVrh10	Phase 1/2	NCT04693598	Recruiting
Krabbe (Early infantile)	AAVHu68	Phase 1/2	NCT04771416	Recruiting
Late Juvenile Metachromatic Leukodystrophy	Lentiviral-HSC GT	Phase 3	NCT04283227	Recruiting
Metachromatic Leukodystrophy	Lentiviral-HSC GT	Phase 2	NCT03392987	Active, not recruiting
Metachromatic Leukodystrophy	Lentiviral-HSC GT	Phase 1/2	NCT01560182	Active, not recruiting
Metachromatic Leukodystrophy	Lentiviral-HSC GT	Phase 1/2	NCT02559830	Recruiting
MPS Type I	rAAV9	Phase 1/2	NCT03580083	Recruiting
MPS Type I	Lentiviral-HSC GT	Phase 1/2	NCT03488394	Active, not recruiting
MPS Type I	ZFN-AAV	Phase 1/2	NCT02702115	Active, not recruiting
MPS Type I	Autologous Plasmablasts	Phase 1/2	NCT04284254	Not yet recruiting
MPS Type II	AAV9	Phase 1/2	NCT04573023	Recruiting
MPS Type II	rAAV9	Phase 1/2	NCT03566043	Recruiting
MPS Type IIa	AAVrh10	Phase 2/3	NCT03612869	Active, not recruiting
MPS Type IIIa	scAAV9	Phase 1/2	NCT04088734	Recruiting
MPS Type IIIa	Lentiviral-HSC GT	Phase 1/2	NCT04201405	Active, not recruiting
MPS Type IIIa	scAAV9	Phase1/2	NCT02716246	Recruiting
MPS Type IIIb	rAAV9	Phase 1/2	NCT03315182	Active, not recruiting
MPS Type IV	AAV2/8	Phase 1/2	NCT03173521	Recruiting
Neuronal Ceroid Lipofuscinosis 3	scAAV9	Phase 1/2	NCT03770572	Active, not recruiting
Neuronal Ceroid Lipofuscinosis 6	scAAV9	Phase 1/2	NCT02725580	Active, not recruiting
Neuronal Ceroid Lipofuscinosis 7	rAAV9	Phase 1	NCT04737460	Recruiting
Pompe (Late-onset)	AAV	Phase 1/2	NCT04093349	Recruiting
Pompe (Late-Onset)	AAV8	Phase 1/2	NCT04174105	Recruiting
Pompe (Late-Onset)	AAV9	Phase 1	NCT02240407	Active, not recruiting
Sandhoff	AAVrh.8	Phase 1	NCT04669535	Recruiting
Tay-Sachs	AAVrh.8	Phase 1	NCT04669535	Recruiting

While AAV-based technologies seek to circumvent an aberrant mutation, other investigational strategies aim to precisely manipulate the patient genome. Currently, meganucleases, zinc finger nucleases, TALENs, and CRISPR/Cas can modulate gene expression by modifying DNA, RNA, or transcription factors. Zinc finger nucleases (ZFNs), engineered proteins comprised of the non-specific cleavage domain of FokI endonuclease and zinc finger proteins[115], showed promising results in hemophilia animal models[116,117], and lead to the initiation of phase 1 clinical trial (NCT02695160). Following this development, ZFNs have been tested in models of Gaucher disease, Fabry disease, MPS type I, and MPS type II, with the administration of AAV8-ZFNs and AAV8 carrying the gene for the relevant lysosomal enzyme to animal models[118-120], and in MSP type I a first-in-human clinical trial (NCT02702115). Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) has also garnered much attention in the field of gene editing. On the therapeutic level, this molecular technology utilizes the RNA-mediated adaptive immune system found in

bacteria to modify, remove, or add genes in vivo[121,122]. An experiment utilizing an intravenously administered novel AAV8 system carrying CRISPR to insert a promoterless α-l-iduronidase (IDUA) cDNA sequence into the albumin locus of hepatocytes in MPS type I-affected neonatal and adult mice resulted in IDUA activity in the brain and reduction of pathology in peripheral organs, as well as notable behavioral improvements, learning ability, and restored memory functionality[123]. A similar strategy was used in models of Tay-Sachs and Sandhoff diseases[124], and in NPC1 mouse model to assess its treatment potential in Niemann-Pick disease type C. The study showed robust base editing in brain, liver, heart, retina, and skeletal muscle cells, leading to a 9.2% increase in lifespan and higher Purkinje neuron survival[125].

Crossing the BBB remains a difficult challenge in searching for effective pharmacological candidates for CNS therapy. Currently, new low molecular weight therapeutic drugs are under development to enhance their interaction with luminal receptors to cross the BBB, like the IgG-IDS fusion protein that showed high penetrance into the brain and spinal cord after intravenously injected in the MSP Type I mouse model[126,127]. Other pharmacological strategies to efficiently access the CNS include nanotechnologies like nanoparticles-based polymers and lipid nanoparticles. Nanoparticles formulated with polylactide-coglycolide (PLGA) showed efficient BBB crossing in MPS type I model and suggest a possible intervention with PLGA loaded with rhGAA (human acid α-glucosidase) [128,129].

As prenatal genetic diagnostics continue to advance in safety and accessibility, the possibility of early diagnosis and intervention of LSDs is becoming a reality. Several studies have now examined in utero ERT[130], *in utero* hematopoietic stem cell therapy[130-132], early gene therapy[133-136], and gene editing[137] for the treatment of various lysosomal storage disorders. Benefits of targeting the disease *in utero* include small fetal size, the tolerant fetal immune system, the presence of highly proliferative and developing stem/progenitor cells, and the potential to treat diseases in which devastating pathology begins prior to birth. The studies tend to target LSDs that have multi-organ dysfunction and *in utero* or perinatal morbidity and mortality, such as MPS type I, MPS type VII, and neuronopathic Gaucher disease. Results from these studies have contributed to the first-in-human fetal ERT clinical trial for the treatment of MPS types I, II, IVa, VI, VII, infantile-onset Pompe disease, Neuronopathic Gaucher Disease (types 2 and 3), and Wolman disease (NCT04532047).

CONCLUSION

Overall, LSDs are multisystemic diseases with a very heterogeneous clinical etiopathology in which progression, complexity, and severity are defined by very diverse factors like genetic background or residual levels of enzymatic activity. New developments, especially in gene therapy, have the potential to improve the efficacy of these interventions by increasing levels of enzymatic expression, both systemically and cellularly, but early diagnosis and pre-symptomatic intervention are essential to ensure therapeutic benefits for those with neurological involvement before irreversible neurological consequences appear. This underscores the need for cost-effective, universal newborn screening by which patients can be rapidly identified and treated.

DECLARATIONS

Authors' contributions

Wrote the manuscript: Munjal V, Thompson-Clarke M, Vignolles-Jeong J, Valencia JA, Samaranch L
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