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Research Article

Review of Lysosomal Storage Diseases; Pathogenesis, Classification and Diagnostic Approach

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Abstract

Lysosomal storage disorders (LSDs) comprise a group of over 80 monogenic disorders. With a few exceptions, the majority are autosomal recessive. LSDs results from the accumulation of incompletely catabolized substrates due to specific enzyme deficiencies in certain metabolic pathways. The accumulated substances gradually impair the function of cell organelles, especially lysosomes. Due to the low incidence of LSDs, they may be overlooked, and patients may go through a diagnostic odyssey. A high index of clinical suspicion is needed to diagnose LSD. The measurement of enzyme activity as a diagnostic tool for LSD is considered the gold standard for diagnosis. Specific individual enzyme activity may be evaluated in leukocytes, plasma, Dried Blood Spot (DBS), or in fibroblasts cultured from a skin biopsy. More recently, two methods were introduced for measuring enzyme activity to screen for several LSDs by multiplexing several enzyme assays in the same sample. These include Digital Microfluidic Fluorometry (DMF) and tandem mass spectrometry. Molecular genetic testing is important in establishing genotype- phenotype correlations, confirmation of a clinical and biochemical diagnosis. It is also important for cases where enzymatic testing is not possible, for genetic counselling and antenatal testing.

Keywords: Mucopolysaccharides; Oligosaccharides; Mucolipidosis; Lipid Storage Disease; Cathepsin Related Disorders; Lsds Caused by Defective Lysosomal Membrane Transport Protein; Lysosomal Glycogen Storage Disease; and Lysosomal Related Organelles.

Introduction

Lysosomal storage disorders (LSDs) comprise a group of over 80 monogenic disorders with the majority being autosomal recessive [1,2]. Exceptions which follow an X-linked pattern of inheritance include Hunter (Mucopolysaccharidosis type-II), Danon and Fabry diseases [3]. Both Danon and Fabry disease may have a late onset presentation and females may present with a milder form of the disease [4].

Due to the low incidence rate of LSDs, they may be overlooked and patients and their families may go through a prolonged diagnostic odyssey [5], until symptoms progress and diagnosis become clearer.

A high index of clinical suspicion is needed to diagnose LSD. History taking is of great importance; as a rule of thumb, LSD should be ruled out in any child with developmental delay and/or regression after an initial period of normal development.

LSDs results from the accumulation of incompletely catabolized substrates due to specific enzyme deficiency in certain metabolic pathway. The accumulated substances impair the function of different cell organelles especially lysosomes. The accumulated substrate is responsible for the characteristic clinical picture for LSDs (e.g. delayed development, organomegaly, skeletal abnormalities and sometimes facial dysmorphism) [6]. Early diagnosis of LSDs is of paramount importance to initiate early management before irreversible organ damage takes place.

Early diagnosis is crucial, especially for LSDs for which effective therapies are available. Newborn screening plays a key role in this process, serving as an important secondary prevention measure. Diagnostic delays are a common challenge for lysosomal storage diseases (LSDs) that manifest in adulthood. Paediatricians are typically more familiar with the severe, "classic" forms of these diseases that present in childhood, whereas adult physicians often overlook the possibility of late-onset variants in their differential diagnosis [7].

Classification of LSDs

Biochemically, LSD can be classified according to the type of accumulated substrate into different categories (e.g. Mucopolysaccharides, Oligosaccharides, Mucolipidosis, Lipid Storage disease, cathepsin related disorders, LSDs caused by defective lysosomal membrane transport protein, lysosomal glycogen storage disease, and Lysosomal-Related Organelles disorders) [8].

Mucopolysaccharidoses (MPS) (Table 1A):

Table 1A: Mucopolysaccharidosis (MPS).

Disease	OMIM	Defective Proteins or Enzymes	Urine metabolites	Biochemical diagnosis	Ref.
MPS IH (Hurler syndrome)	607014	Alpha-L-iduronidase	Dermatan sulphate, heparin sulphate	Measurement of enzymatic activity in plasma, leukocytes, fibroblasts, trophoblastic cells, amniocytes, or in DBS. Enzyme activity in chorionic villi and amniotic fluid cells can be measured for prenatal diagnosis	99-110
MPS IS (Scheie syndrome)	607016				
MPS IH/S (Hurler-Scheie syndrome)	607015				
MPS II (Hunter syndrome)	309900	Iduronate sulfate sulfatase	Dermatan sulphate, heparin sulphate		
MPS III (Sanfilippo syndrome)			Heparin sulphate		
Type IIIA	252900	Heparan N-sulfatase			
Type IIIB	252920	Alpha-N-acetylglucosaminidase			
Type IIIC	252930	Acetyl CoA:alpha-glucosaminide acetyltransferase			
Type IIID	252940	N-acetylglucosaminine-6-sulfatase			
MPS IV (Morquio syndrome			Keratin sulphate; in IVB, also chondroitin 6-sulfate		
Type IVA	253000	Galactosamine-6-sulfate sulfatase			
Type IVB	253010	Beta-galactosidase			
MPS VI (Maroteaux-Lamy syndrome)	253200	N-Acetylgalactosamine-4sulfatase (arylsulfatase B)	Dermatan sulphate		
MPS VII (Sly syndrome)	253220	Beta-glucuronidase	Dermatan sulphate, heparan sulphate, chondroitin 4-sulfate, chondroitin 6-sulfate		
MPS IX (hyaluronidase deficiency)	601492	Hyaluronidase	None		

MPS is a group of disorders that results from defect in one of the enzymes that metabolize Glycosaminoglycans (GAGs). The latter are disaccharide repeats that may be present in sulphated or non-sulphated forms and play a crucial role in cell signalling, proliferation, adhesion, anticoagulation, and collagen fibres integrity. Five forms of GAGs are present: Keratan sulphate (KS), Heparan sulphate (HS), Dermatan sulphate (DS), Chondroitin sulphate (CS) and hyaluronic acid (HA). Eleven enzyme defects in the lysosomal degradation of Glycosaminoglycans have been identified and each result in a specific MPS disorder (MPS I, MPS II, MPS IIIA, MPS IIIB, MPS IIIC, MPS IIID, MPS VII, and MPS IX).

MPS I is caused by the lack of the enzyme alpha-L-iduronidase. The severe form is called MPS I H, (Hurler syndrome, OMIM 607014). The other subtypes of MPS I are MPS I H-S (Hurler-Scheie syndrome OMIM 607015) and MPS I S (Scheie syndrome OMIM 607016).

MPS II (Hunter syndrome OMIM 309900) is caused by a deficient iduronate-2-sulfatase.

MPS III (Sanfilippo syndrome) has four subtypes (A, B, C and D), all of which are inherited in an autosomal recessive manner. Each subtype is caused by a deficiency in a different enzyme in the catabolic pathway for heparan sulphate. Type A (OMIM 252900): heparan-N-sulfatase, type B (OMIM 252920): alpha-N-acetylglucosaminidase, type C (OMIM 252930): alpha-glucosaminide N-acetyltransferase, and type D (OMIM 252940): N-acetylglucosamine-6-sulfate sulfatase.

MPS IV (Morquio syndrome) result from deficient enzymes N-acetylgalactosamine-6-sulfatase, MPS IVA (Morquio A disease OMIM 253000) or beta-galactosidase MPS IVB (Morquio B disease, OMIM 253010)

MPS VI (Maroteaux–Lamy syndrome, OMIM 253200) is caused by lack of the enzyme N-acetylgalactosamine 4-sulfatase.

MPS VII (Sly syndrome, OMIM 253220) is caused by lack of the enzyme beta-D-glucuronidase.

MPS IX (Natowicz syndrome, OMIM 601492), is caused by lack of the enzyme hyaluronidase.

Recently, a newly characterised lysosomal hydrolase Arylsulfatase K (ARSK, OMIM 610 011)) deficiency disorder has been described [9]. ARSK is involved in the removal of 2- O- sulfate group from 2- sulfoglucuronate. Affected individuals share similar clinical picture of other MPS, that include developmental delay, mild facial dysmorphism and increased excretion of GAGs in urine specifically mild elevation of Dermatan sulphate 9.

Oligosaccharidosis (Table 1B):

Table 1B: Oligosaccharidosis.

Disease	OMIM	Defective Proteins or Enzymes	Biochemical diagnosis	Ref.
Sialidosis*	256550	Neuraminidase 1 (sialidase)	Increased urinary sialyloligosaccharides, demonstration of deficient neuraminidase activity in leukocytes or, in cultured fibroblasts	111
Type I (cherry-red macular spot-myoclonus syndrome, mild form)				
Type II (congenital, infantile, juvenile, and childhood forms)				
Sialolipidosis** (phospholipidosis)	252650	Transport defect in the lysosomal pathway	Elevated plasma gastrin concentration	112
Galactosialidosis† (Goldberg syndrome, combined neuraminidase, and beta-galactosidase deficiency)	256540	Protective protein/cathepsin A (PPCA)	Elevated sialyloligosaccharides but no free sialic acid	113
Alpha-mannosidosis, type I (severe) or II (mild)	248500	Alpha-D-mannosidase	Mannose-rich oligosaccharides in urine, Measuring acid alpha-mannosidase activity in leukocytes	114
Beta-mannosidosis	248510	Beta-D-mannosidase	Increased Mannosyl-N-acetylglucosamine in urine, Reduced Beta-mannosidase activity	115
Fucosidosis	230000	Alpha-L-fucosidase	Increased urinary excretion of fucosylated oligosaccharides, reduced α-L-fucosidase activity in the blood	116
Aspartylglucosaminuria	208400	N-Aspartylglucosaminidase	Increased urinary excretion of aspartylglucosamine, assaying the activity of AGA enzyme in blood, fibroblasts, amniocytes or trophoblasts	117
Winchester syndrome	277950	Metalloproteinase-2	None	

Schindler disease		N-Acetyl-galactosaminidase	Increased urinary secretion of Oligosaccharides and O-linked sialopeptides, NAGA enzyme assessed on leukocyte, blood plasma or cultured lymphoblasts or fibroblasts	118 119
Type I (infantile severe form)	609241			
Type II (Kanzaki disease, adult-onset form)	609242			
Type III (intermediate form)	609241			

*Sialidosis, also known as mucolipidosis type I (ML I)

**Sialolipidosis is also known as mucolipidosis type IV (ML IV)

†Galactosialidosis is also classified as a Cathepsin related disorders

Oligosaccharides are a class of carbohydrates that are compose of 2–10 monosaccharide joined together via an O-glycosidic or N-glycosidic bonds. Examples of oligosaccharides include sucrose, maltose, fucose, manose, lactose, rafinose, fructofuranose and trehalose. Oligosaccharidosis results from the accumulation of different oligosaccharides in tissues due to specific enzyme deficiency in their metabolic pathway. Oligosaccharidosis include α-mannosidosis, β-Mannosidosis, fucosidosis, Sialidosis (also known as Mucolipidosis type I), galactosialidosis, Aspartylglucosaminuria, Schindler disease, and Torg-Winchester syndrome [10]. Clinically, oligosaccharidosis resembles MPS. Clinical features include severe neurological symptoms that may present early in life.

Galactosialidosis (see below Cathepsin related disorders)

α-mannosidosis (OMIM 248500) is an example of oligosaccharidosis that results from α-mannosidase enzyme deficiency with accumulation of mannose in tissues, while Schindler disease (Alpha-N-acetylgalactosaminidase deficiency) is a very LSD that is characterized by the accumulation of oligosaccharides as well as glycosphingolipids and glycoproteins in tissues.

β-mannosidosis (OMIM 248510) results from the deficiency of the enzyme lysosomal β-mannosidase which is encoded by MANBA gene. Deficient enzyme activity results in the storage of disaccharides with β-mannosyl residues

Fucosidosis (OMIM 230000) results from deficiency of the enzyme α-L-fucosidase that is required for the breakdown of fucose [11].

Aspartylglucosaminuria (AGU) (OMIM 208400) is caused by loss of the activity of the enzyme aspartylglucosaminidase (AGA) resulting in the accumulation of aspartylglucosamine in tissues.

Torg-Winchester syndrome (OMIM 259600) is part of a spectrum of diseases called multicentric osteolysis nodulosis and arthropathy spectrum (MONA). MONA spectrum disorders are caused by mutations in the MMP2 or MMP14 genes.

Schindler Disease (OMIM 609241) is due to lack of activity of the enzyme α-N-acetylglucosaminidase. Deficient enzyme activity results in the accumulation of glycopeptides, glycosphingolipids, and oligosaccharides. Clinically, it can be divided into three phenotypes: Type I is an infantile-onset neuroaxonal dystrophy; type II, is an adult-onset form, and type III is an intermediate disorder with mild to moderate neurologic manifestations.

Mucolipidosis (ML) (Table 1C):

Table 1C: Mucolipidosis (ML).

Disease	OMIM	Defective Proteins or Enzymes	Biochemical diagnosis	Ref.
ML I (Sialidosis)	256550	α-N-acetyl neuraminidase deficiency	Increased urinary sialyloligosaccharides, demonstration of deficient neuraminidase activity in leukocytes or, in cultured fibroblasts. Sialidosis is differentiated from galactosialidosis by analyzing the enzymatic activity of β-galactosidase which should be normal.	120
Type I (cherry-red macular spot-myoclonus syndrome, mild form)				
Type II (congenital, infantile, juvenile, and childhood forms)				
ML II (I-cell disease)	252500	N-Acetylglucosaminyl-1-phosphotransfeerase catalytic subunit	Lysosomal enzyme test	121
ML III (pseudo-Hurler polydystrophy)		N-Acetylglucosaminyl-1-phosphotransfeerase	Lysosomal enzyme tests, assaying GlcNAc-1-phosphotransferase activity in fibroblasts	122
Type III-A	252600	Catalytic subunit		
Type III-C	252605	Substrate-recognition subunit		
ML IV	252650	Transient receptor potential channel mucolipin-1	Electron microscopic demonstration of storage organelles	123

*Sialidosis, also known as mucolipidosis type I (ML I)

The Mucolipidoses are progressive disorders that share many features with MPS including developmental delay, organomegaly, and dysostosis multiplex [12]. In Mucolipidosis the lysosomal enzymes are mis-localized to the extracellular space and intracellular concentrations are very low. Mucolipidoses can be sub-divided into ML I, II, III and IV.

Mucolipidosis Type I (ML I) (also known as sialidosis, OMIM 256550) is caused by the deficiency of α-N-acetyl neuraminidase/ sialidase enzyme due to a mutation in the neuraminidase gene (NEU1) [13]. Neuraminidase enzyme deficiency results in the accumulation of sialic acid rich oligosaccharides. Clinically, sialidosis manifests as two phenotypes: Sialidosis type I (cherry-red spot myoclonus syndrome) which is a mild form, and Sialidosis type II which is the severe form [14]. Sialidosis type II is further subdivided into congenital, infantile, and juvenile subtypes.

Mucolipidosis Type II (ML II) (OMIM 252500), also named I-cell disease. ML II is caused by deficiency of the lysosomal-enzyme; N-acetylglucosamine-1-phosphotransferase (GlcNAc-1-phosphotransferase) that is responsible for tagging lysosomal hydrolase enzymes to mannose 6-phosphate to be transported across the lysosomal membrane [15,16]. ML II may present with corneal clouding, organomegaly, dystosis multiplex, facial dysmorphism, kyphoscoliosis, lumbar gibbus. It may also result in severe neurological deterioration.

Mucolipidosis Type III (ML III) type III alpha/beta (OMIM 252600), and type III gamma (OMIM 252605): This type is also called Pseudo-Hurler polydystrophy. ML III is also caused by deficiency of GlcNAc-1-phophotransferase [17]. Clinically, most patients have preserved intellectual function but largely present with skeletal manifestation [18].

Mucolipidosis type IV (MLIV) (OMIM 252650) is caused by mutation in MCOLN1 gene. Patients present with neurological manifestation, achlorhydria, anaemia, renal impairment without dysmorphic features [18].

Lipid Storage disease/Sphingolipidosis (Table 1D):

Table 1D: Lipid Storage disease/Sphingolipidoses.

Disease	OMIM	Defective Proteins or Enzymes	Biochemical diagnosis	Ref.
GM1 gangliosidosis		Ganglioside beta-galactosidase	Assay of beta-galactosidase activity	124
Type I (infantile type)	230500			
Type II (juvenile type)	230600			
Type III (adult type)	230650			
GM2 gangliosidosis			Assay beta-hexosaminidase activity	124
Type I (Tay-Sachs disease)	272800	Beta-hexosaminidase A		
Type II (Sandhoff disease)*	268800	Beta-hexosaminidase B		
GM2 activator protein deficiency (Tay-Sachs disease AB variant, GM2A)	272750	GM2 activator protein	Normal HEXA A and HEXA B activity	125
Niemann-Pick disease				
Type A	257200		Quantifying ASM activity in circulating leukocytes or cultured skin fibroblasts	126
Type B	607616			
Type C1/Type D	257220	NPC intracellular cholesterol transporter 1	Cholestane-3β, 5α, 6β-triol (C-triol) and 7-ketocholesterol (7-KC), plasma lyso-sphingomyelin (lyso-SM)	22 92 127
Type C2	607625	NPC intracellular cholesterol transporter 2		
Gaucher disease		Glucosylceramide beta-glucosidase	Assaying glucocerebrosidase activity in peripheral blood mononuclear cells, or cultured fibroblasts. <10-15% of mean normal activity is consistent of the diagnosis of Gaucher disease	75 128
Type I (adult or chronic form)	230800			
Type II (infantile form)	230900			
Type III (juvenile form, Norrbottnian type)	231000			

Farber disease (lipogranulomatosis)	228000	Acid ceramidase	Measurement of the activity of the enzyme acid ceramidase in peripheral blood leukocytes, cultured lymphoid cells, cultured skin fibroblasts or amniocytes.	129 130
Fabry disease	301500	Trihexosylceramide alpha galactosidase	Assay of α-GAL A activity in leukocytes or plasma, and/or detection of GL3 deposition in tissue biopsies	131 132
Metachromatic leukodystrophy	250100	Arylsulfatase A	ARSA enzymatic activity in skin fibroblasts, leukocytes, and urine. Urinary sulfatides is suggested a biomarker for monitoring MLD.	25 133 134 135 136
Mucosulfatidosis (multiple sulfatase deficiency	272200	Sulfatase-modifying factor-1	Assaying sulfatase activity, Elevated urinary glycosaminoglycan and sulfatides levels	137 138
Krabbe disease	245200	Galactosylceramide beta-galactosidase	Measurement of galactocerebrosidase activity, measurement of psychosine using liquid chromatography MS/MS. Galactosylsphingosine (GalSph, psychosine) has been suggested for diagnosis and monitoring of Krabbe disease and proposed to help differentiate infantile from later onset, pseudodeficiency in addition to monitoring progression before and after treatment.	139 140 141 142
Lysosomal acid lipase deficiency · Wolman disease · Cholesteryl ester storage disease (CESD)	278000	Lysosomal acid lipase	Imaging, biopsy, histology, determination of LAL enzyme activity in a DBS, and molecular analysis of the LIPA gene. Plasma biomarkers include liver enzymes, lipid profiles, and oxysterols such as 7-keto-cholesterol and cholestane-3β,5α,6β-triol	143
Cerebrotendinous xanthomatosis (cholestanol lipidosis)	213700	Sterol 27-hydroxylase	Increased plasma cholestanol and increased urinary excretion of bile alcohols. Determination of plasma ketostetrol bile acid precursors (7-α-hydroxy-4-cholesten-3-one, 5-α-cholestanol)	145
Neuronal ceroid lipofuscinosis				
CLN1, Santavuori-Haltia disease, Infantile Batten disease	256730	Palmitoyl-protein thioesterase-1	Skin biopsy, enzyme levels measurement in blood, dried blood spots, and cultured fibroblasts, electron microscopy studies. The diagnosis can be confirmed by genetic testing	146-150
CLN2, Jansky-Bielschowsky disease	204500	tripeptidyl peptidase 1		
Late infantile form				
Juvenile form (CLN3, Batten disease, Vogt-Spielmeyer disease)	607042	Lysosomal transmembrane CLN3 protein		
CLN4, Adult form, Kufs disease	611203	Cysteine string protein alpha (CSPα)		
Variant late infantile form, Finnish type (CLN5)	608102	Lysosomal transmembrane CLN5 protein		
Variant late infantile form (CLN6)	601780	Transmembrane CLN6 protein		
Ceroid lipofuscinosis, neuronal, 7	610951	MFSD8 protein		
Ceroid lipofuscinosis, neuronal, 8	600143	Transmembrane CLN8 protein		
CLN9	609055	—		
Ceroid lipofuscinosis, neuronal, 10	610127	CLN10/CTS		
Ceroid lipofuscinosis, neuronal, 11	614706	Progranulin		
CLN12	-	ATP13A2		
Ceroid lipofuscinosis, neuronal, 13 (Kufs type)	615362	autofluorescent material		

*An important diagnostic finding in Sandhoff disease is that both HEXA A activity and HEXA B activity are decreased, in addition to total hexosaminidase activity.

CLN3, CLN5, CLN6, CLN8 are also considered a Lysosomal Transport Defects

Sphingolipidoses are caused by defective lysosomal degradation of sphingolipids as a result of mutations in the genes encoding lysosomal enzymes [19]. Sphingolipidosis may also develop secondary to defect in activator proteins involved in degradation pathways of sphingolipids (Saposins defects) or due to impairment in the trafficking and fusion in the lysosomal endocytic system. There are more than [10] genetic Sphingolipidosis subtypes these include [20]: gangliosidosis type I, II (GM1 and GM2), galactosialidosis, metachromatic leukodystrophy, Acid sphingomyelinase deficiency (ASMD), Niemann-Pick disease (NPA/B/C), Gaucher disease, Fabry disease, Krabbe disease, Tay-Sachs disease, Multiple Sulfatase deficiency, and Farber Disease. Enzyme deficiency results in accumulation of high quantities of glycosphingolipids and phosphosphingolipids in various cell types.

GM1 gangliosidosis (β-galactosidase-1 deficiency) (OMIM 230500) results from a defect in the metabolism of gangliosides caused by deficiency of β-gangliosidase resulting in central nervous system (CNS) and liver manifestations. Three types have been described; The most severe early infantile is GM1, where affected babies present with hepatosplenomegaly, skeletal/neurologic manifestations, and cherry-red spots in the eyes. Late infantile GM1 may present between 1 and 3 years of age with seizures and neurological manifestations, while the less progressive adult form of GM1 may present later in life with neurological manifestations, corneal opacifications and hypotonia.

GM2 gangliosidosis include Tay–Sachs disease (TSD) (OMIM 272800); Sandhoff disease, (OMIM 268800); and GM2 activator protein deficiency (OMIM 272750). Tay Sachs disease and its more severe form, Sandhoff disease, result from a deficiency of the enzyme, β-Hexosaminidase caused by bilallelic pathogenic variants in the genes HEXA (Tay-Sachs) or HEXB (Sandhoff) disease, coding the alpha or beta subunits of the enzyme. Clinical phenotypes present as a continuum of neurological manifestations and delayed development based on the amount of residual β-hexosaminidase activity [21].

Niemann-Pick disease is divided into four subtypes: type A, type B, type C1, and type C2.

Niemann-Pick type A (NPA) (OMIM 257200) and Niemann-Pick type B (NPB) (OMIM 607616) are caused by biallelic pathogenic variants in the sphingomyelin phosphodiesterase 1 (SMPD1) gene resulting in deficient activity of the enzyme acid sphingomyelinase (ASM; "types A or B").

Niemann-Pick type C (NPC) is caused by mutation in the NPC1 (OMIM 257220) or NPC2 (OMIM 607625) genes leading to the deficiency of an intracellular lipid trafficking proteins [22]. NPC is characterized by the build-up of endo-lysosomal cholesterol and glycosphingolipids. Toxic accumulation of un-esterified cholesterol, glucosylceramide, and gangliosides in the late endosomal/lysosomal compartment of the cell results in cellular and organ damage.

Gaucher disease is caused by pathogenic variants in the GBA gene that codes for the β--glucocerebrosidase enzyme that metabolizes glucoceramides. Gaucher disease is classified as non-neuronopathic or type 1 (OMIM 230800) and neuronopathic or type 2 (OMIM 230900) or type 3 (OMIM 231000) forms. Patients with Gaucher disease may have hepatosplenomegaly, pulmonary disease. dermatologic changes, cytopenia and anaemia secondary to bone marrow suppression as a result of accumulation of glucosylceramide [23]. Four types of Gaucher disease have been described; a Perinatal lethal form that is characterised by extrapyramidal signs. Acute infantile/childhood type that is characterised by bulbar, extrapyramidal signs and cognitive impairment. A childhood/early juvenile form that is characterized by oculomotor involvement, seizure and progressive myoclonic epilepsy. The fourth type is the adult form that has no neurological involvement but is characterized mainly by organomegaly and cytopenia.

Farber disease (Farber lipogranulomatosis, OMIM 22800) is caused by mutations within N-acylsphingosine amidohydrolase (ASAH1) encoding for acid ceramidase that hydrolyses ceramide into sphingosine and free fatty acid. Absent enzyme activity leads to the accumulation of ceramide in tissues [24]. Clinically, patients present with a triad of progressive joint deformity, subcutaneous lipogranulomatosis, and hoarseness of voice due to laryngeal involvement. Neurological and respiratory manifestations may develop as the disease progresses.

Fabry disease (OMIM 301500) is an X-linked LSD that is caused by mutations in the GLA gene leading to deficient α-galactosidase A enzyme with progressive accumulation of globotriaosylceramide (Gb3 or GL-3 and related glycosphingolipids (galabiosylceramide) within lysosomes. The whole mark of Fabry disease is the progressive deterioration in renal function and cardiomyopathy that develop later in affected males. Females are carriers and might develop a milder form of the disease.

Metachromatic leukodystrophy (MLD), (OMIM 250100): Is a progressive neurodegenerative lysosomal disorder caused by a deficiency in the enzyme Arylsulfatase A (ARSA). This results in accumulation of sulfatides in lysosomes. MLD is classified into late infantile, juvenile, and adult types according to the residual enzyme activity and age of onset [25]. Affected patients present with progressive neurological manifestation, seizures, and developmental delay.

Multiple sulfatase deficiency (MSD) (OMIM 272200) is caused by the deficiency of formylglycine-generating enzyme, that is encoded by the Sulfatase Modifying Factor 1 (SUMF1) gene. There are seventeen types of sulfatases, nine of them may be associated with MSD [26]. Enzyme deficiency leads to the accumulation of sulfatides, sphingolipids, sulphated glycosaminoglycans, and steroid sulphates in tissues. Clinically affected patients may present with neurological manifestations, dysmorphism, hepatosplenomegaly and delayed development.

Krabbe disease (Globoid cell leukodystrophy) (OMIM 245200) is caused by mutations in the galactocerebrosidase (GALC) gene leading to the deficiency of the enzyme galactocerebrosidase, that hydrolyses psychosines and galactosylceramide. The absence of galactocerebrosidase leads to the toxic accumulation of galactosylsphingosine (psychosine) in oligodendrocytes and Schwann cells with progressive neurological and cognitive deterioration and seizures [27].

Lysosomal acid lipase deficiency (LAL-D; OMIM 278000) is a severe autosomal recessive disorder involving multiple organs. Lysosomal acid lipase (LAL) is responsible for the breakdown of lysosomal cholesterol esters and triacylglycerols. LAL deficiency is caused by mutations in the LIPA gene, and this results in the accumulation of cholesterol esters and triacylglycerols in the lysosomes. LAL-D is classified into two types: the early onset more severe infantile form (Wolman Disease) and the late onset childhood/adult types (cholesterol esters, storage disease (CESD). The early onset form results in more severe clinical manifestations of hepatosplenomagly, hyperlipidaemia, impaired liver function and malabsorption due to diarrhoea, vomiting and adrenal calcifications with short life expectancy. The late onset CESD is characterised by hyperlipidaemia, hepatic impairment, premature atherosclerosis, and hepatic steatosis.

Cerebrotendinous xanthomatosis (cholestanol lipidosis-CTX) (OMIM 213700) is caused by a bile acid synthetic defect that is caused by mutations in the CYP27A1 gene that encodes the mitochondrial enzyme sterol 27-hydroxylase. Decreased enzyme activity leads to accumulation of large quantities of cholestanol in tissue.

Neuronal ceroid lipofuscinoses: Several subtypes of NCL have been identified, however the common hallmark of all of them is the accumulation of intracellular ceroid lipofuscin.

CLN1 PPT1 (Santavuori–Haltia disease / infantile NCL) (OMIM 256730)

CLN2/LINCL TPP1 (Jansky–Bielschowsky disease / late infantile NCL) (OMIM 204500).

CLN3Type 3 (Batten–Spielmeyer–Vogt disease / juvenile NCL) (OMIM 204200)

CLN4 (Kufs disease / adult NCL) (OMIM 162350)

CLN5 Ceroid Lipofuscinosis, Neuronal, 5 (OMIM 256731); CLN5 Intracellular Trafficking Protein (OMIM 608102)

CLN6 (Late infantile variant) (OMIM 606725)

CLN7 (Mutations in CLN7/MFSD8) (OMIM: 610951)

CLN8 (Mutations in the CLN8 gene cause CLN8 disease) (OMIM 600143)

CLN9 (CLDN9 gene encodes a member of the claudin family) (OMIM 615799)

CLN10 Ceroid Lipofuscinosis, Neuronal, 10; (OMIM 610127) (See below)

CLN11 Ceroid Lipofuscinosis, Neuronal, 11 (OMIM 614706)

CLN12 is caused by mutations in the ATP13A2 gene.

CLN13 (Ceroid lipofuscinosis, neuronal, 13 (Kufs type) (OMIM 615362) is due to mutation in the CTSF gene (Cathepsin F)

CLN14 (CLN14 disease)

Cathepsin related disorders (Table 1E):

Table 1E: Cathepsin related disorders.

Disease	OMIM	Defective Proteins or Enzymes	Diagnosis	Ref.
Galactosialidosis	256540	Lysosomal protective protein deficiency, deficiency of cathepsin A, PPCA deficiency	Sialyloligosaccharidurıa	151
Haim-Munk syndrome (HMS)	245010	Cathepsin C (dipeptidyl peptidase I), a lysosomal protease	Diagnosis is based on clinical manifestations, skin biopsy, measurement of enzymatic activity. Diagnosis is confirmed by genetic testing.	152 153
Papillon-Lefevre syndrome (PLS)	245000			
Periodontitis 1, juvenile	170650			
Cathepsin D deficiency	610127	See above (Ceroid lipofuscinosis, neuronal)		
Cathepsin F deficiency	615362	See above (Ceroid lipofuscinosis, neuronal, 13 (Kufs type)		
Pycnodysostosis	265800	Cathepsin K deficiency	None	

Lysosomal cathepsins are proteolytic enzymes that play an important role in lysosomal metabolism. Over or under expression of lysosomal cathepsins have been associated with specific disorders according to the affected lysosomal cathepsin protease [28]. Till the time of writing this review, 15 humans lysosomal cathepsin proteases have been identified with 5 specific disorders described in human due to genetic deficiency of specific lysosomal cathepsin proteases [13].

Galactosialidosis (OMIM 256540) (Lysosomal protective protein deficiency, deficiency of cathepsin A, PPCA deficiency) is caused by mutation in the CTSA gene which results in impaired functioning of cathepsin A. CTSA plays a crucial protective role to stabilize the protein complex made up by β-galactosidase (β-GAL) and neuroamidase-1 (NEU1), thereby protecting these two glycosidases from lysosomal degradation [29]. Biochemically there is increased urinary excretion of sialated oligopsaccharides with three clinical subtypes identified. An early infantile that may present in utero with foetal hydrops, visceromegaly, cherry red spots in retina and death [30]. A late infantile with organomeagly, corneal clouding and cardiac impairment. The late onset juvenile or adult form may present with spinal deformities, ataxia, myoclonus, and neurological deterioration. Galactosialidosis is also considered a sialic acid disorder.

Cathepsin C deficiency (OMIM 602365) is due to mutation in the CTSC gene. Mutations in the CTSC gene may cause Papillon-Lefevre syndrome (PALS) or Haim-Munk syndrome (HMS). The characteristic features include ectodermal dysplasia, palmoplantar keratoderma with early-onset aggressive periodontitis. [31-33].

Cathepsin D deficiency (OMIM 116840): Cathepsin D is a lysosomal aspartic proteinase of the pepsin family. Genetic variants within the CTSD gene have been linked to Parkinson's and Alzheimer's disease as well as neuronal ceroid lipofuscinosis type-10 (NCL10) [34]. Sever CLN type10 can manifests with epileptic seizures, microcephaly, and death within hours to weeks after birth. Patients with residual CTSD activity develop late infantile, juvenile, or adult type 10 CLN with milder neuropathological phenotypes [35].

Cathepsin F deficiency (OMIM 603539): Mutation in cathepsin F gene results in the development of the adult-onset type 13 CLN disease [36]. Patients may present with neurological deterioration and dementia [37].

Pycnodysostosis (OMIM 265800) is a rare autosomal recessive LSD and is caused by mutations in the gene encoding CTSK [38]. Cathepsin K is a lysosomal protease that is abundant in the osteoclasts. Affected patients may present with osteoporosis, clavicular abnormalities, short stature and delayed closure of fontanels, scull sutures and dental abnormalities.

Inherited LSDs caused by defective lysosomal membrane transport protein (Table 1F):

Table 1F: Lysosomal Transport Defects.

Disease	OMIM	Defective Proteins or Enzymes	Biochemical diagnosis	Ref.
Cystinosis				
Infantile nephropathic form	219800	Cystinosin (lysosomal cystine transporter)	Clinical features including renal Fanconi syndrome, increased cystine levels in leucocytes. Diagnosis is made by detecting elevated intracellular cystine content. Diagnosis can be confirmed by genetic analysis of the cystinosin gene. Prenatal diagnosis is possible within 24 hours of sampling by the quantitative measurement of cystine in uncultured chorionic villi (CVS) or by the measurement of 35S-labeled cystine in a cultured amniocytes or CVS by thin layer chromatography and visual inspection of autoradiographs of the chromatograms for cystine.	154 155
Late-onset juvenile or adolescent form	219900			
Adult non-nephropathic form	219750			
Salla disease	604369	H+/sialic acid (acidic hexoses) symport	Increased urinary free sialic acid. Measurement of free sialic acid in fibroblasts lysosomes	156
Infantile sialic acid storage disorder	269920	Sodium phosphate cotransporter		
Finnish type (Salla disease)	604369	Sodium phosphate cotransporter		
French type	269921	UDP-N-acetylglucosamine-2-epimerase/N-acetylmannosamine kinase		

Cystinosis (cystine transporter deficiency) is a rare autosomal recessive LSD caused by mutations in the CTNS gene that is responsible for the synthesis of cystanosin (the lysosomal cytine transporter protein). Deficiency of cystansoin results in the accumulation of cystine crystals within the lysosomes of different tissues (especially the kidneys and cornea). Cystinosis has three main forms: Cystinosis, nephropathic; CTNS (OMIM 219800), cystinosis, adult nonnephropathic (OMIM 219750), cystinosis, late-onset juvenile or adolescent nephropathic Type (OMIM 219900) [39]. Cystinosis is the most common cause of inherited Fanconi syndrome in children [40]. Clinically affected patients present with polyuria, polydipsia, normal anion gap hypercholremic metabolic acidosis, hypophosphataemic rickets due to loss of phosphate in urine and progressive deterioration of renal functions due lysosomal deposition of cystine crystals with the development of End Stage Renal Disease by the first decade of life. Affected patient may have photophobia as well due to deposition of cystine crystals in the cornea. The Nephropathic Jeuvenile form represents about 5% of cystinosis cases. Affected patients have mild clinical manifestation, mild proteinuria and may present later with nephrotic syndrome like presentation with less progressive impairment in renal functions. The adult, non-nephropathic cystonsis is mainly an ocular form of cystinosis that is characterised by photophobia due to corneal cystine accumulation.

Infantile sialic acid storage disease (ISSD) (OMIM 269920) results from a mutation in the SLC17A5 gene that encodes the sialin protein that is responsible for the transport of sialin across the lysosomal membrane [41].

Salla disease (OMIM 604369) is an autosomal recessive neurodegenerative disorder results from impaired sialic acid transport across lysosomal membranes due to mutations in the SLC17A5 gene. It has a high carrier rate in the Salla region of Finland [42]. It is characterised by psychomotor delay, spasticity, athetosis, organomegaly and seizures. Clinical manifestations are secondary to the accumulation of silaic acid in tissue with increase excretion and urine and high level in CSF and other body fluids.

Sialuria (OMIM 269921) is an extremely rare form of sialic acid disorders. It results from failure of CMP-Neu5Ac to feedback inhibit UDP-N-acetylglucosamine (UDP-GlcNAc) 2-epimerase [43,44]. Clinical features include developmental delay, mildly coarse features, hepatosplenomegaly, and microcytic anaemia. Sialuria is characterized by deposition of sialic acid in tissue and increased urinary excretion of free sialic acid.

Lysosomal glycogen storage disease (Table 1G):

Table 1G: Lysosomal glycogen storage disease.

Disease	OMIM	Defective Proteins or Enzymes	Biochemical diagnosis	Ref.
Glycogen storage disease type 2 (Pompe disease)	232300	Deficiency of acid alpha-glucosidase (acid maltase)	GAA enzyme assay in cultured fibroblasts or muscle	157
Danon disease	300257	Lysosomal associated membrane protein-2 (LAMP-2)	Histological and/or electron microscopy findings of autophagic vacuoles containing glycogen granule in skeletal and cardiac muscle fibres	158

Glycogen storage disease type II (Pompe disease) (OMIM 232300) is an autosomal recessively inherited metabolic disorder due to mutations in the GAA (alpha glucosidase/Acid maltase enzyme gene with prominent involvement of cardiac, smooth, and skeletal muscles [45]. GAA is responsible for the breakdown of glycogen into glucose that is used for energy production. Pompe disease is classified into two types; an early onset (infantile form) is caused by the complete or near complete deficiency of GAA enzyme with symptoms presenting as early as first month of life. Clinical presentation may include, hypotonia, cardiomyopathy, poor feeding and difficulty in breathing. A late onset (juvenile/adult) results from partial deficiency of GAA and can begin as early as the first decade of childhood or well into adulthood. Muscle weakness progresses to death from respiratory failure may occur.

Glycogen storage disease type IIb (Danon disease) (OMIM 300257) is due to the deficiency of LAMP-2 (Lysosomal-Associated Membrane Protein 2). It is characterised by cardiomyopathy, muscle weakness, and sometimes with intellectual deficit [46].

Disorders of Lysosomal-Related Organelles (LROs) (Table 1H):

Table 1H: Disorders of Lysosomal-Related Organelles (LROs).

Disease	OMIM	Defective Proteins or Enzymes	Clinical/Biochemical diagnosis	Ref.
Hermansky-Pudlak Syndrome (HPS)	203300	defective lysosome-related organelles	Lack of platelet dense bodies	159
Chediak-Higashi Syndrome (CHS)	214500	lysosomal trafficking regulator protein	Affected patients usually have neutropenia, decreased natural killer–cell cytotoxicity during lymphocytes subset analysis and hypergammaglobulinemia. A characteristic feature for CHS is the presence of coalescent granules of lysosomes in neutrophils and other cell types in peripheral blood and during bone marrow examination. Definitive diagnosis is by molecular analysis to identify pathogenic variants in the LYST or CHS1.	160

Griscelli syndrome type 1	214450	myosin Va, (part of a group of proteins called unconventional myosins)	Clinical features, demonstration of the presence of skin granulomas and NK cytotoxic dysfunction	161
Griscelli syndrome type 2	607624	Rab27a protein		
Griscelli syndrome type 3	609227	melanophilin	Clinical features, examination of peripheral blood film, skin, hair, and schwann cells biopsies	162
Hereditary platelet delta (δ)-storage pool deficiency	185050	Fewer dense granules in platelets	Clinical features, bleeding assessment, transmission electron microscopy to visualize the fewer dense granules in platelets	163
Wiskott-Aldrich syndrome	301000	Wiskott-Aldrich syndrome protein (WASp)	Thrombocytopenia associated with small platelet volume is a consistent finding in patients with mutations of the WASP gene	164
Thrombocytopenia Absent Radius syndrome	274000	RNA-binding motif protein 8A	Clinical features, thrombocytopenia, and molecular genetic testing	165

These disorders result from defect in an integral membrane protein rather than a defect in certain lysosomal enzyme. Examples of these conditions include platelet-granules disorders. Defective platelet function defects may be secondary to Lysosomal related organelles defect. This can be caused by a defect in the number of platelets granules or failure of platelets granules secretion upon stimulation. Several disorders have been described to be associated with a defect in either dense- or alpha-granules. Dense granules are a lysosome-related organelle that are localised to platelets. The platelet defect in dense-granule disorders causes a bleeding diathesis with easy bruising and bleeding tendency. Lysosomal related organelles disorders that may have a dense platelets granules specific defect with normal platelets count include: Hermansky-Pudlak Syndrome (HPS), Chediak-Higashi Syndrome and idiopathic dense-granule deficiency.

Hermansky-Pudlak Syndrome (HPS) is caused by mutations in eleven different genes. Hermansky-Pudlak Syndrome 1; HPS1 (OMIM 203300), HPS2 (OMIM 608233), HPS3 (OMIM 614072), HPS4 (OMIM 614073), HPS5 (OMIM 614074), HPS6 (OMIM 614075), HPS7 (OMIM 614076), HPS8 (OMIM 614077), HPS9 (OMIM 614171), HPS10 (OMIM 617050), HPS11 (OMIM 619172). It not fully understood how mutations in the HPS genes lead to the specific clinical manifestations that include oculocutaneous albinism, normal platelets count but defective dense granules, that causes systemic complications with accumulation of ceroid-lipofuchsin in tissues [47].

Chediak-Higashi Syndrome (CHS) (OMIM 214500) results from a mutation in the LYST or the CHS1 genes [48]. Defective genes lead to disruption of the storage and secretory functions of lysosomal granules of different cell types including leukocytes, fibroblasts, platelets, and melanocytes. CHS manifest with oculocutaneous albinism, immunodeficiency, bleeding tendency and neurological dysfunction. An early-onset form and an attenuated, later-onset form have been described.

Storage Pool Deficiency (SPD) (OMIM 185050) is characterized by combined alpha/delta- platelets granules deficiency [49]. The platelet abnormality in SPD is very similar to HPS, even though SPD patients have normal pigmentation and no evidence of ceroid-lipofuchsin storage. Structurally platelets are normal apart from the marked deficiency/absence of dense bodies. There is marked deficiency of adenine nucleotides and serotonin however it is less profound than in HPS. The mode of inheritance appears to be autosomal dominant.

Wiskott-Aldrich Syndrome (WAS) (OMIM 301000) is an X-linked disorder caused by mutations in the WASP gene caused by mutations in WASP gene that encodes a protein that regulates cellular actin cytoskeleton rearrangement. WAS platelets have markedly reduced delta granules, alpha-granules, and mitochondria. It is associated with microthrombocytopenia, immunodeficiency and eczema [50]. Wiskott-Aldrich Syndrome 2; WAS2 (OMIM 614493) is an autosomal recessive. It is characterized by recurrent infections in infancy, thrombocytopenia and eczema.

Thrombocytopenia Absent Radius syndrome (TAR) (OMIM 274000) is caused by compound heterozygosity for a null allele and an RBM8A hypomorphic allele. It is characterized by thrombocytopenia and bilateral absence of the radii in the presence of thumbs [51]. Defects in platelet granules are reported in a subset of TAR patients.

Griscelli syndrome (GS)/ Griscelli-Preunieras Syndrome is a rare LRO disorder that results from defective melanin containing organelles (melanosomes) that are responsible for hair and skin pigmentation. GS is characterized by a silvery-grey sheen of the hair and hypopigmentation of the skin. GS is classified into 3 subtypes. GS1 type GS1 (OMIM 214450) is caused by mutations in the MYO5A gene. It is associated with neurological manifestations and possibly delayed development. GS2 (OMIM 607624) results from mutations in the RAB27A gene. It is accompanied with immunologic impairment while GS 3 (OMIM 609227) is due to mutations in Melanophilin (MLPH). It is characterised by cutaneous manifestations and silvery-grey sheen of hair.

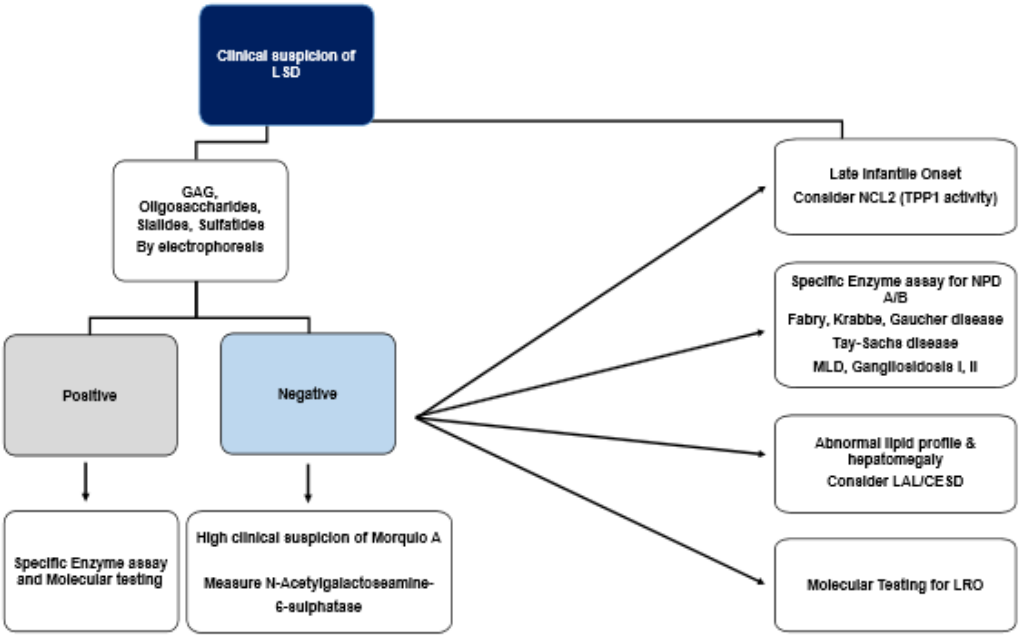
Diagnosis of LSDs

LSDs can affect various organs and systems, leading to a wide range of clinical manifestations. Early and accurate diagnosis is crucial for managing these conditions, as many LSDs are progressive and can lead to significant morbidity and mortality if left untreated. The diagnostic process typically involves a combination of clinical evaluation, biochemical testing, genetic analysis, and imaging studies [52]. Advances in newborn screening and enzyme replacement therapies have improved early detection and treatment outcomes, making timely diagnosis essential for optimizing patient care.

The first step in the diagnosis of LSDs starts with the detection of the accumulated substrates in biological fluids. Biochemical diagnosis of LSDs usually involves the collection of a set of biological samples. Sample type include, a urine sample, a dried blood spot (DBS) card and two 3-5 ml EDTA samples. Urine should be kept frozen until processed for Glycosaminoglycans quantitation and possible GAGs, oligosaccharides, sialic acid identification and fractionation. The DBS card sample should be allowed to properly air dry for 2 hours, then kept in the freezer (–20 °C) in plastic bag till used for the enzyme assays. The first EDTA sample is kept at 4°C and leucocytes are promptly separated as soon as possible and used for specific enzyme assays. Ideally, EDTA sample for leucocyte enzyme assay should be processed with 24 hours of collection. The second EDTA tube is used to separate DNA that will be stored at -80°C and used for molecular confirmation of diagnosis.

Urine Analysis

A tiered approach to the diagnosis of LSD has been suggested (Figure 1). Urine samples are analysed to detect increased excretion of specific substrates (e.g. glycosaminoglycans/ GAG, oligosaccharides, sulfatides, Ceramides). The increase in excretion of GAGs can initially be detected using the dimethyl methylene blue (DMB) colorimetric assay where the dye 1,9-DMB combines with GAGs to form complex molecules. This reaction causes a colour change from blue to pink which can be measured spectrophotometrically [53]. Figure 2 summaries the different GAG profiles in urine samples of patients with MPS. Once an increased urinary excretion of certain substrate (e.g. GAGs) is confirmed, the second-tier testing is conducted for the separation/fractionation of individual glycosaminoglycan. Thin layer Chromatography or 2-Dimentional electrophoresis maybe used to detect increased excretion of oligosaccharides, sulfatides and sialic acid [54]. When there is a clinical suspicion of MPS, but no elevated GAGs, e.g. urine GAGs may be within normal limits in MPSIV or MPSIII, a second-tier testing is required.



LSD: Lysosomal Storage Diseases, GAG: Glycosaminoglycans CLN2: Neuronal Ceroid Lipofuscinosis Type 2 (Batten disease), TPP1: Tripeptidyl Peptidase 1, NPD A/B: Niemann-Pick disease A/B, MLD: Metachromatic Leukodystrophy, LAL: Lysosomal Acid Lipase, CESD: Cholesterol Ester Storage Disease, LRO: Lysosomal-Related Organelles.

Figure 1: Diagnostic Pathway for Lysosomal Storage Diseases.

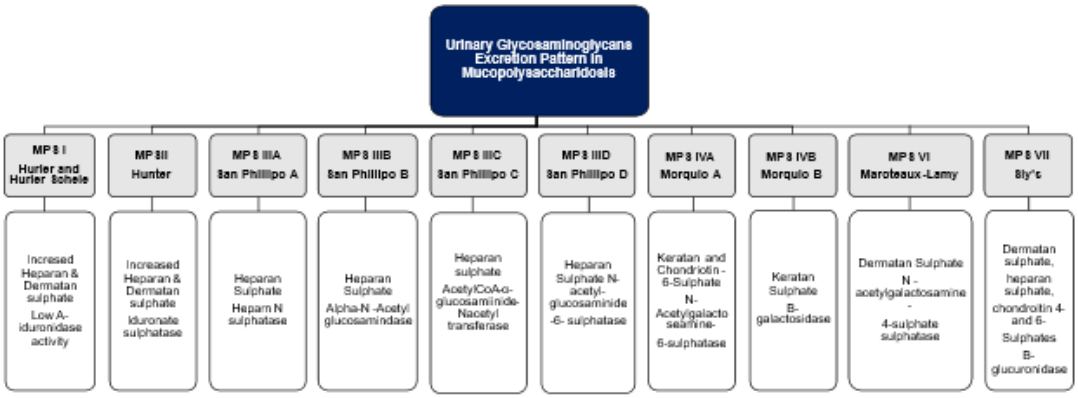


Figure 2: Urinary Glycosaminoglycans Excretion Pattern in Mucopolysaccharidosis.

Enzymatic Assay

Measurement of lysosomal enzyme activity plays an important role in screening and the diagnosis of LSDs [55]. Enzyme activity may be evaluated in plasma, DBS or skin biopsy used for fibroblast culture. The later has the disadvantages of being invasive, long turnaround time with the risk of culture failure or contamination. Alternatively, enzyme activity can be determined in leucocytes sample, however the leucocytes should be extracted within 24-72 hours of blood venepuncture. Recently an accurate and specific Liquid Chromatography Mass Spectrometry (LC-MS) method for measurement of individual glycosaminoglycans in serum or urine has been developed [56]. Once a suggestive profile is detected in urine that points to certain enzyme deficiency, measurement of the enzyme activity is arranged. The latter is considered the gold standard diagnostic tool for LSDs. Two methods were introduced for measuring enzyme activity to screen for several LSD through multiplexing several enzyme assays in the same test. Those; include Digital Microfluidic Fluorometry (DMF) and Tandem Mass Spectrometry. DMF developed by Advanced Liquid Logic, Inc. (now Baebies, Inc. Durham, NC, USA) uses synthetic 4-methylumbelliferone (4-MU) as synthetic substrate to assess different enzyme activities. [57]. The second method for enzyme assay is tandem mass spectrometry-based assay. The assay is based on the extraction of different enzymes from DBS then incubation with different enzyme specific internal standards and six substrates in a single buffer. This is followed by liquid–liquid extraction and Tandem Mass flow injection analysis (FIA) [58,59].

Although measurement of the enzyme activity is the gold standard for diagnosis of LSD, the results of the enzyme activity should be interpreted carefully in certain disorders. For example, although N-acetylgalactosamine-6-sulfatase (GALNS) is found in MPS IVA (Morquio A syndrome), it can be reduced in other disorders such as multiple sulfatase deficiency. Thus, finding a normal level of one or two of the sulfatase enzymes removes this confusion [60].

Molecular Diagnosis

Molecular diagnosis is important in cases where enzyme assay cannot be performed, and diagnosis was based upon the specific clinical picture. For example, whenever TPP1 enzyme activity cannot be measured in a suspected case of NCL2, molecular detection of two pathogenic variants in trans establishes the diagnosis [61]. Furthermore, molecular confirmation may be used to guide therapy and to decide on personalized management approach and prognostication. Some mutations may carry a better prognosis than others; for example (p.Ser633Trp) variant in MPS-I usually results in a milder form of the disease [62].

Although molecular testing markedly improved the diagnosis of LSD, the interpretation of molecular/genetic testing may not be always straight forward depending on the type of the variants detected. A diagnostic uncertainty may arise when the variant detected is of unknown significance or a compound heterozygous for a pathogenic variant and another variant of unknown significance. In this case, a trio testing may be needed to check the potentially causative variant in the parents as well and whether the variant is present in Cis or Trans in the affected patient. Pitfalls of molecular testing include: a lack of sufficiently large ethnicity-specific genetic datasets with the risk of labelling a pathogenic variant as a VUS. Furthermore, a poor genotype correlation with the residual enzyme activity is noted in several LSD. Other pitfalls of molecular testing include the detection of pseudodeficiency alleles. Pseudodeficiency is a characteristic phenomenon in LSD especially some MPS (MPS I, IIIB, IVB, VI, and VII), Arylsulphatase A in MLD and β-Galactocerebrosidase in Krabbe disease where a certain lysosomal hydrolase shows a low enzyme activity in vitro while the patient is not presenting clinically. This phenomenon is attributed to the presence of polymorphic genetic variants that affect some of lysosomal hydrolases. In cases of suspected Pseudo deficiency, biochemical tests (GAG assays) and/or specific enzyme assay using natural substrate may elucidate the diagnosis11 [63-65].

Monitoring of the progression of LSDs

Urinary level of certain GAGs has been suggested as an indirect biomarker of MPS progression. It was found that HS and DS decrease after initiation of ERT in MPSI patients, but do not correlate with clinical outcomes [66,67]. Several studies suggested the potential

use of serum and urine level of KS measurements as a biomarker for assessing clinical severity of MPS type IVA, and IVB at an early stage and monitoring therapeutic effects [68]. Serum heparin-cofactor II-thrombin complex (HCII-T) has been used to monitor long-term therapeutic efficacy of ERT in MPS I, II and MPS VI patients [69-70]. Fibroblast growth factor-2 (FGF-2) is a molecule with high affinity for Heparin Sulphate (HS). High levels of FGF-2 may indicate increased HS [71]. Inflammatory cytokines IL-6 and Polymerization of Pyrin domain (PYD) were found to be associated with the progression of skeletal disease in MPSI patients [72]. Biochemical measurement of galactose-containing oligosaccharides in the urine may also be used as a useful marker to monitor GM1 gangliosidosis disease severity. Plasma Oxysterols (cholestane-3β, 5α, 6β-triol); lyso-SM-509 and lyso-sphingomyelin can be used to screen for NPC and to monitor disease progression. They can serve as sensitive markers for acid sphingomyelinase deficiency and is elevated in ASMD and NPC [22,43]. More recently, plasma palmitoyl phosphocholineserine (PPCS, formerly lyso-sphingomyelin-509) has replaced plasma oxysterol (cholestane-3β,5α,6β-triol) as a first-line screen for NPC [73]. PPCS has improved clinical performance when compared with C-triol: including improved clinical specificity, fewer unnecessary re-bleeds, no false positives in co-existing cholestatic jaundice and improved clinical sensitivity. Available biomarkers for monitoring Gaucher disease include serum level of chitotriosidase, glucosyl sphingosine and ferritin. Tartrate-resistant acid phosphatase (TRAP) and angiotensin converting enzyme (ACE) are less used because of their poor specificity [74,75]. Chitotriosidase is a plasma enzyme that is considered as an inflammatory marker and a general LSD storage marker. Increased Chitotiosidase level are indication of macrophage activation that is increased during LSD in a trial to clear the accumulated Lysosomal enzymes substrates. It has been suggested as marker to monitor therapy in Gaucher disease [76,77]. Plasma and urinary level of GB3/LysoGB3 and LysoGb1 has been used to monitor Fabry's [78,79] diseases and to assess disease progression [80]. A potential biomarker, globotriaosylsphingosine (lyso-Gb3), is elevated in the plasma of affected males and to a lesser extent in adult females with classical FD. Monitoring lyso-Gb3 levels appears promising for assessing enzyme replacement therapy, with lyso-Gb3 serving as an independent risk factor for cerebrovascular white matter lesions in male patients and correlating with overall disease severity in females [81-83]. Lyso-Gb3 could be a valuable biomarker, as demonstrated by elevated plasma levels in Fabry's patients receiving enzyme replacement therapy [84]. Recently an LCMS method for the determination of sphingolipids in biological fluids have been developed to assess lysosphingolipids (LysoSLs), the N-deacetylated lyso-forms of glycosphingolipids that have been found to be markedly elevated and specific marker for patients with sphingolipidosis [85-88].

Newborn screening of LSDs

Newborn screening for LSDs was first started in early seventies with a very successful, plasma-based community carrier screening program for Tay–Sachs disease. If screen positive, couples could undergo amniocentesis or chorionic villus sampling to identify affected foetuses. Screening within the Ashkenazi Jewish decreased the number of children born with Tay–Sachs disease in the United States from 60 per year before 1970 to 3–5 per year by 1983 (a 90% reduction in the incidence within this population) [89]. Pompe disease is a progressive, debilitating, and often life-threatening disease. Early treatment of affected infants is crucial in improving prognosis. The availability of alpha glucosidase as an enzyme replacement therapy for Pompe disease, made it a suitable candidate disorder for newborn screening. Soon after, several treatments became available for other metabolic disorders. This made newborn screening possible of several LSD [90,91]. Newborn screening (NBS) for MLD is currently being piloted due to the availability of a mass spectrometry-based screening method that measures C16:0 sulfatides in Dried Blood Spot and the advances in gene therapy combined with haematopoietic transplantation. Quantification of the bile acid in DBS, could be used for a newborn screen for NPC [92]. The Recommended Uniform Screening Panel (RUSP) have been expanded recently to include 6 Mucopolysaccharidosis disorders in newborn screening programs. On the contrary, ethical concerns about the universal screening for LSD were raised, as some diseases might have a milder course with an adult-onset and may not require treatment for decades [93,94].

NBS can sometimes yield uncertain findings, raising significant ethical concerns, including diagnostic uncertainty, premature treatment risks, and the psychological burden on families [95]. Key ethical issues include variant interpretation, identifying carriers and benign variants, unnecessary treatments, high medical costs, and managing late-onset disorders with continuous follow-up [96]. New York State began screening all newborns for Krabbe disease in 2006. Nearly two million infants were screened, and only five were diagnosed with early infantile Krabbe disease. The NY outcomes were unexpectedly poor [97]. Of these, three infants died—two due to complications from hematopoietic stem cell transplantation (HSCT) and one from the progression of untreated disease. Two children who underwent HSCT developed moderate to severe developmental delays. Additionally, significant number of asymptomatic children at the time of screening are considered at moderate or high risk for developing later-onset Krabbe disease [98]. The risks, benefits [99-110], and effectiveness of early intervention need to be carefully weighed [111-130], and ongoing assessments are essential to guide the implementation of screening programs and ensure that they provide real value to affected children [131-165].

Conclusion

Diagnosis of LSDs requires a high index of clinical suspicion and should be in any child with a growth arrest after a period of normal development. Laboratory diagnosis involves a tier wise approach that starts with the detection of the increased excretion of certain substrate, identification of disease specific profile, detection of

reduced enzyme activity and finally molecular confirmation of the presence of pathogenic variants. New-born screening for LSD is increasingly recognized in different countries. The use of newborn screening to detect LSDs and the subsequent decision to perform aggressive treatments such as HSCT involves a complex interplay of medical, ethical, and practical considerations. These issues highlight the need for careful consideration and balanced decision-making to ensure that the benefits of NBS outweigh its potential harms.

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