

Common and Uncommon Pathogenic Cascades in Lysosomal Storage Diseases^{*[5]}

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Lysosomal storage diseases (LSDs), of which about 50 are known, are caused by the defective activity of lysosomal proteins, resulting in accumulation of unmetabolized substrates. As a result, a variety of pathogenic cascades are activated such as altered calcium homeostasis, oxidative stress, inflammation, altered lipid trafficking, autophagy, endoplasmic reticulum stress, and autoimmune responses. Some of these pathways are common to many LSDs, whereas others are only altered in a subset of LSDs. We now review how these cascades impact upon LSD pathology and suggest how intervention in the pathways may lead to novel therapeutic approaches.

All eukaryotic cells contain lysosomes, membrane-bound organelles that contain a range of acid hydrolases such as proteases, glycosidases, sulfatases, phosphatases, and lipases. Impairment of the activity of these enzymes leads to progressive accumulation of unmetabolized substrates, resulting in the monogenic disorders known as lysosomal storage diseases (LSDs).² Although monogenic diseases are simple in terms of the causative gene defect, the biochemical and cellular cascade of events that ensue is highly complex (1).

LSDs are typically inherited as autosomal recessive traits and occur at a collective frequency of ~1:5000 live births. Over 50 LSDs are known; they can be caused by defects in soluble lysosomal enzymes, in non-enzymatic lysosomal proteins (soluble or membrane-bound), or in non-lysosomal proteins that impinge upon lysosomal function. The degree of residual function of the defective protein influences the age of symptom onset. Patients null or almost null for a given protein present symptoms *in utero* or in early infancy, whereas milder muta-

tions lead to juvenile or adult onset disease (1). The majority of LSDs involve storage in both the central nervous system (CNS) and visceral tissues. CNS pathology is a common hallmark of LSDs, and LSDs are the commonest cause of pediatric neurodegenerative disease.

LSD classification is usually based on the biochemical nature of the accumulating substrate. Thus, disorders in which sphingolipids are the primary accumulating material are classified as sphingolipidoses, those in which mucopolysaccharides (*i.e.* glycosaminoglycans) accumulate are known as mucopolysaccharidoses (MPS), and oligosaccharides accumulate in the oligosaccharidoses. However, classification according to the accumulating substrate is often misleading, as is the case with the neuronal ceroid lipofuscinoses (NCLs), classically characterized as one disease family, based on the common intracellular accumulation of ceroid lipopigments and of subunit c of the mitochondrial ATP synthase. It is, however, now known that each of the NCL subtypes is caused by defects in one or other of nine seemingly unrelated CLN proteins (2).

Despite the distinctive types of storage material in different LSDs, they share many common biochemical, cellular, and clinical features. Thus, advances in understanding one particular disease can provide insight into other specific LSDs or into LSDs in general. We now summarize the known biochemical and cellular pathways that are altered in either human LSDs or animal models of LSDs and attempt to classify the pathways as either “common” or “uncommon.” It should be stressed that classification of a pathway as either common or uncommon is based to a large extent on the availability, or not, of systematic studies of each particular pathway in each specific LSD. Furthermore, the classification is often hampered by the different cell types used in different studies. Many studies have been performed in patient fibroblasts, but LSD fibroblasts often bear little resemblance to the major cell types that are affected in each disease because fibroblasts may not accumulate substrates to the same extent as the clinically relevant cell types. Thus, the value of comparing, for instance, studies in fibroblasts with studies in cultured neurons or in brain tissue is not always readily apparent. This is exemplified using different Gaucher disease models, where unfolded protein response (UPR) activation and abnormal antioxidant response were found in Gaucher disease fibroblasts (3, 4) but were not observed in bone marrow mesenchymal stromal cells (5) or in neurons (6). Great care must therefore be taken when drawing conclusions from studies on the same disease but using different cell types.

Nevertheless, this classification (Table 1 and [supplemental Table 1](#)) should be of use to researchers attempting to elucidate the relative significance of the biochemical and cell biological pathways associated with a specific disease, which is vital if effective novel therapies are to be developed (7). As each LSD is individually rare, targeting common clinical intervention points with agents that could treat multiple diseases is particularly attractive. This is even more relevant in light of the rather limited therapeutic options available at present for many of the

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[5] The on-line version of this article (available at <http://www.jbc.org>) contains [supplemental Table 1](#).

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² The abbreviations used are: LSD, lysosomal storage disease; CNS, central nervous system; ER, endoplasmic reticulum; GlcCer, glucosylceramide; MPS, mucopolysaccharidoses; NCL, neuronal ceroid lipofuscinoses; NPC, Niemann-Pick type C; ROS, reactive oxygen species; UPR, unfolded protein response; LacCer, lactosylceramide.

TABLE 1
Common and uncommon pathways altered in the sphingolipidoses

A plus sign indicates that the pathway was altered, and a minus sign indicates that the pathway was unaltered; the absence of a sign or of a reference (given in parentheses) indicates that no data are available. A complete version of this table, including pathways altered in the other LSDs discussed in this review, can be found in [supplemental Table 1](#).

Disease	Defective protein	Main storage material	Oxidative stress	ER stress and UPR	Altered calcium homeostasis	Autophagy	Inflammation	Altered lipid trafficking
GM1 gangliosidosis	β -Galactosidase	GM1 ganglioside	+ (3)	+ (16, 17)	+ (16, 17)	+ (41)	+ (20)	+ (49)
GM2 gangliosidosis (Sandhoff)	β -Hexosaminidase A and B	GM2 ganglioside and related glycolipids	+ (20)	+ (13)	+ (13)	+ (13, 39)	+ (20)	+ (49)
GM2 gangliosidosis (Tay-Sachs)	β -Hexosaminidase A	GM2 ganglioside and related glycolipids	+ (3)	+ (3)	+ (20)	—	+ (20)	+ (49)
Gaucher	β -Glucosidase or saposin C activator	GlcCer	+ (4, 50)	— (6)	+ (11, 12, 51)	— (39)	+ (52, 53)	+ (54)
Fabry	α -Galactosidase A	Globotriaosylceramide and blood group B substances	+ (21)	—	—	—	—	+ (49)
Niemann-Pick Type A	Sphingomyelinase	Sphingomyelin	—	—	+ (14)	—	+ (55)	+ (49)
Niemann-Pick Type C ^a	NPC1 and -2	Cholesterol and sphingolipids	+ (22, 23, 31)	+ (3)	+ (10)	+ (37–39, 56, 57)	+ (31)	+ (49)
Metachromatic leukodystrophy	Arylsulfatase A or saposin B activator	Sulfated glycolipids and GM1 ganglioside	—	—	—	—	+ (58)	+ (49)
Globoid cell leukodystrophy (Krabbe)	Galactocerebroside β -galactosidase	Galactosylceramide	—	—	—	—	+ (59)	+ (49)
Farber	Ceramidase	Ceramide	—	—	—	—	+ (60)	— (49)

^a NPC disease is not a primary sphingolipidosis but is listed in the table because NPC cells accumulate significant levels of sphingolipids as secondary storage materials.

diseases, particularly the rarer diseases, which are usually characterized by a distinct and fatal CNS phenotype.

Accumulation of Secondary Metabolites

In many LSDs, there is accumulation of secondary metabolites, such as gangliosides GM2 and GM3 (8) and cholesterol (9), that are unrelated to the primary genetic defect. In Niemann-Pick C (NPC) disease, secondary GM2 and GM3 accumulation may be the result of a trafficking defect that is downstream to defects in lysosomal calcium homeostasis (10). Cholesterol storage in the sphingolipidoses is probably related to the tight membrane association and modes of regulation of these lipids, although a precise mechanism is lacking to explain the concomitant changes in sphingolipid and cholesterol levels in the LSDs.

Calcium Homeostasis

Defective intracellular calcium signaling has emerged as a key common pathway in LSDs. In retrospect, this is perhaps not surprising because calcium plays vital roles in regulating a variety of cellular events, with impaired calcium homeostasis leading to endoplasmic reticulum (ER) stress, oxidative stress, and cell death. However, the mechanism leading to impaired calcium homeostasis is different in each LSD, depending on the mode of interaction of accumulating substrates with specific calcium channels or pumps, or on other upstream events. Altered calcium homeostasis can be classified according to the organelle in which defective calcium signaling is observed.

Increased calcium release from the ER occurs in models of neuronal forms of Gaucher disease due to overactivation of the ER calcium channel, the ryanodine receptor (11); glucosylceramide (GlcCer), the lipid that accumulates in Gaucher disease, directly modulates the ryanodine receptor (12). In Sandhoff disease (a GM2 gangliosidosis) (13) and in Niemann-Pick A disease (14), elevated cytosolic calcium is due to a reduction in the rate of calcium uptake by the sarco/endoplasmic reticulum Ca^{2+} -ATPase (SERCA); in the case of Sandhoff disease, ganglioside GM2 modulates SERCA activity *in vitro* (13), which depends on an exposed sialic acid residue on GM2 (15). ER calcium store depletion also occurs in the GM1 gangliosidosis (16), where GM1 interacts with the phosphorylated form of the inositol 1,4,5-trisphosphate-gated calcium release channel (17).

Mitochondrial calcium homeostasis is altered in at least two LSDs. In a neuronal model of the GM1 gangliosidosis, GM1 accumulation in a glycosphingolipid-enriched fraction of mitochondria-associated ER membranes results in elevated mitochondrial membrane permeabilization, opening of the permeability transition pore, and activation of the mitochondrial apoptotic pathway (17). In skin fibroblasts from mucopolysaccharidosis type IV patients, mitochondrial fragmentation and decreased mitochondrial calcium buffering were observed, leading to oversensitivity of the cells to proapoptotic effects induced by calcium-mobilizing agonists (18), although calcium-dependent apoptosis appeared to depend on activation of a non-mitochondrial caspase 8 pathway.

Lysosomal calcium is altered in NPC1 disease. NPC1 cells display a major reduction in lysosomal calcium stores as a result of sphingosine storage, resulting in defective endocytic fusion

and trafficking, which subsequently induces cholesterol, sphingomyelin, and glycosphingolipid storage. Compensating for the alterations in lysosomal calcium levels by elevating cytosolic calcium reverses the NPC1 phenotype and prolongs survival of NPC1 mice (10).

Finally, in a mouse model of the juvenile form of NCL, calcium homeostasis is altered by modulation of a plasma membrane voltage-gated calcium channel. Elevated levels of the Gnb1 protein were observed; Gnb1 is a β 1 subunit of the G protein complex, which negatively regulates N-type voltage-gated calcium channels. Upon inhibition of N-type calcium channels, recovery from depolarization was lower in Cln3-deficient neurons, resulting in a prolonged period of higher intracellular calcium (19).

Oxidative Stress and Free Radicals

Accumulating evidence suggests that reactive oxygen species (ROS) play a pivotal role and are perhaps common mediators (3) of cell death in many LSDs. Thus, up-regulation of apurinic endonuclease 1 (APE1) (a protein that repairs oxidative DNA damage) was observed in Gaucher fibroblasts (4) (but not in Gaucher bone marrow mesenchymal stromal cells (5)). In the GM1 and GM2 gangliosidoses, inducible nitric oxide synthase and nitrotyrosine were elevated in activated microglia/macrophages (20), and ROS was elevated in Fabry disease models (21). Gene microarray analysis from NPC1 fibroblasts was consistent with enhanced oxidative stress (22), and elevated ROS and lipid peroxidation rendered the fibroblasts more susceptible to cell death after an acute oxidative insult (23). In MPSIIIB, enhanced oxidative stress resulted in protein, lipid, and DNA oxidation (24), and an oxidative imbalance was found in MPSI (25). In NCLs, elevated ROS and superoxide dismutase levels were suggested to be downstream to ER stress (26), a significant increase in manganese-dependent superoxide dismutase activity was detected in fibroblasts and brain extracts from CLN6 sheep (27), and increased expression of 4-hydroxynonenal was detected in late infant and juvenile forms of NCL (28).

The central role that oxidative stress plays in integrating other cellular pathways and stresses suggests that it is most likely activated in LSDs as a secondary biochemical pathway, rather than as a direct result of accumulation of the primary substrate. Moreover, the possible role of oxidative stress may be of real significance in delineating LSD pathology, particularly as oxidative stress plays a central role in other better studied neurodegenerative conditions.

Inflammation

Although the LSDs involve storage of self-components, a common host response is the inappropriate activation of the immune system, resulting in chronic inflammation. The exact mechanisms leading to immune activation are unknown but probably reflect altered signaling pathways in response to storage. For instance, in type 1 Gaucher disease, which lacks CNS involvement, the main storage cell types are macrophages, which are found throughout the body but are particularly prevalent in the liver, spleen, and bone marrow. GlcCer storage in macrophages leads to macrophage activation and release of multiple cytokines and to the release of the chitinase, chitotri-

osidase, which serves as a useful clinical biomarker for this disease (29).

In LSDs with CNS pathology, brain inflammation is a common and universal feature. In the brain, macrophage lineage cells are represented by microglia, which respond to trauma and disease by activating the inflammatory response (30). In healthy individuals, microglia are in a resting state, but if infection or trauma is sensed, they undergo a wave of self-limiting activation until the disease/damage episode is resolved (30). Upon substrate accumulation in LSDs, an inflammatory response is triggered that is not self-limiting, and once triggered, it progressively increases in parallel with the storage burden (20). Numerous studies in multiple LSDs indicate that the inflammatory process contributes to pathogenesis (see, for instance, Refs. 20 and 31). One example is the GM1 and GM2 gangliosidoses, where activation of both CNS and peripheral inflammation predates the onset of clinical signs and involves elevation of multiple proinflammatory cytokines (20).

It is likely that lysosomal storage causes immune activation by different molecular mechanisms depending on where storage occurs and on the biochemical nature of the stored molecules. However, once activated, there is convergence in the development of chronic inflammation. Despite the fact that inflammation is a downstream event in the pathogenic cascade, it may nevertheless be a target for adjunctive therapy in multiple LSDs. Thus, when a mouse model of Sandhoff disease was treated with non-steroidal anti-inflammatory drugs or crossed with the MIP1 α (chemokine CCL3) knock-out mouse to prevent peripheral immune cell recruitment to the brain, clinical benefit resulted (32, 33). The mouse model of NPC1 also benefits from non-steroidal anti-inflammatory therapy (31).

Lipid Trafficking

Altered lipid trafficking has been shown in some LSDs but not in others. A fluorescent analogue of lactosylceramide (BODIPY-LacCer) accumulates in the Golgi apparatus in normal fibroblasts after its addition from exogenous sources but accumulates in late endosomes and lysosomes in fibroblasts obtained from some LSD patients (*i.e.* GM1 gangliosidosis, GM2 gangliosidosis, prosaposin deficiency, metachromatic leukodystrophy, MPSIV, Fabry disease, Niemann-Pick disease (types A, B, and C), and Krabbe disease) (34). In contrast, no endosomal accumulation of BODIPY-LacCer was observed in other LSD fibroblasts, such as Pompe disease, Hunter disease, NCL, Farber, and Gaucher disease, although altered BODIPY-LacCer trafficking was observed in a chemically induced Gaucher disease model (35), suggesting that the lack of change in lipid trafficking in Gaucher fibroblasts may have been due to the lack of GlcCer accumulation in fibroblasts. The mechanism by which lipid trafficking is altered in some LSDs is unknown but is related to changes in cholesterol levels because depletion of cholesterol in LSDs restored normal BODIPY-LacCer trafficking.

Autophagy

Autophagy is a vacuolar, self-digesting mechanism responsible for removal of long lived proteins and damaged organelles. Autophagosomes fuse with lysosomes for degradation of their

cargo by lysosomal hydrolases. Aside from its role in survival during stress conditions, autophagy can also serve as a programmed cell death mechanism, with impairment of autophagosome-lysosome fusion or overinduction of autophagy leading to autophagosome accumulation and cell death.

Impaired autophagy contributes to the pathogenesis of several neurodegenerative diseases. Due to the vital role of the lysosome in autophagy, this pathway is an obvious candidate in LSD pathogenesis (36) and has been extensively studied, particularly in NPC. In brains obtained from *Npc1*^{-/-} mice, levels of the autophagy marker, LC3-II, were significantly elevated, and autophagic vacuole-like structures were seen (37). LC3-II elevation was observed upon treatment of cells with the cholesterol-altering drug, U18666A, in NPC1-deficient Chinese hamster ovary cells (38) and in NPC1-deficient human fibroblasts (39). These changes were associated with increased expression of beclin-1, a protein that is activated during autophagy induction.

Activation of autophagy has also been observed in other LSDs such as in NPC2, Sandhoff disease, multiple sulfatase deficiency and MPSIIIA (40), GM1 gangliosidosis (41), NCLs, and Pompe disease (42, 43). The latter studies demonstrated that autophagic buildup had a profound effect on the endocytic pathway. Defective LAMP-2 (the integral lysosomal membrane protein defective in Danon disease) causes accumulation of autophagosomes in many tissues, leading to cardiomyopathy and myopathy (44). Hence, impaired autophagy seems to play a role in many LSDs, although the underlying mechanism differs between diseases. In the GM1 gangliosidosis, in NPC and in NCL, the impairment is due to autophagy overactivation, whereas in other LSDs (*i.e.* multiple sulfatase deficiency and MPSIIIA), autophagosome-lysosome fusion is defective.

Alterations in autophagy are often directly related to altered intracellular lipid trafficking (see above). Thus, autophagy appears normal (as measured by LC3-II and beclin-1 levels) in Gaucher disease fibroblasts, which traffic sphingolipids normally (39). Clearly, further studies on Gaucher cells are needed, as are studies on the relationship between lipid trafficking and autophagy activation in LSDs, to definitely determine the commonality of this pathway to LSD pathogenesis.

ER Stress and the UPR

In the ER, secretory and transmembrane proteins fold into their native conformations and undergo post-translational modifications. When these functions are impaired, misfolded proteins accumulate in the ER lumen and activate the UPR, which can initiate apoptosis. Unfolded protein accumulation can occur in response to changes in the ER environment, including glucose starvation, reducing agents, and depletion of ER calcium stores. Because calcium homeostasis is altered in LSDs, this pathway could also be potentially involved in LSD pathology.

Activation of the UPR was recently suggested to be a common mediator of apoptosis in LSDs, based on studies showing UPR activation in fibroblasts from a wide variety of LSDs (3). However, a systematic study examining various neuronal Gaucher mouse models found no evidence for UPR activa-

tion (6). UPR activation was shown in mouse tissues and in neurospheres obtained from GM1 gangliosidosis mice (16), in the brain and in cultured cells from infantile NCL (45), but was not observed in spinal cord tissue from a mouse model of sialidosis (16).

Autoimmune Disease

Autoantibody responses to storage material is not a commonly reported feature of the LSDs. However, in one report, anti-GM2 autoantibodies were detected in a mouse model of Sandhoff disease; crossing Sandhoff mice with mice deficient in Fc receptor γ prolonged their survival, suggesting an antibody-mediated component in this disease (46). Autoimmune responses to molecules in the brain that are not themselves stored in the disorder are also uncommon in LSDs, although there is some evidence for this occurring in the MPSIIIB mouse (47). Similar findings were reported in Batten disease (48) with autoantibodies present against multiple CNS components. The possible role of autoimmune disease merits more detailed and methodical study.

Concluding Comments

We have surveyed the different biochemical and cellular cascades that are altered in LSDs. For some diseases, wide ranging and systematic studies have been performed, whereas for other diseases, scant data are available. This reflects to some extent the relatively low frequency of each individual disease in the population. However, more advances in understanding the downstream events affected in the LSDs are likely to come about in the years ahead, with the realization that these monogenic diseases present unique opportunities for studying the basic biology of some of the cellular pathways discussed above. The convergence of increased understanding of the underlying biology of LSD pathology with progress in modes of therapeutic intervention should result in a fruitful period of research on LSDs and in the development of new therapies.

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