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Lysosomal Storage Diseases

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Abstract

Lysosomal storage diseases are a group of inherited and acquired disorders. They are characterized by interruption of recycling of cellular and extracellular molecules. Clinically, they are presented as developmental and neurological symptoms similar to other inherited and acquired disorders. This article reviews the function of lysosomes, the current mechanisms that cause the interruption of recycling, the consequences that are manifested clinically, and the methods to diagnose these disorders.

Keywords

lysosome function, the etiology of the diseases, clinical manifestations, diagnostic tools

The lysosome is the primary disposal and recycling center of cells. Macromolecules of intracellular and extracellular origin are metabolized in the lysosome. This provides cells with amino acids, fatty acids, nucleic acids, and carbohydrate residues for reuse in cellular synthesis.[1] For example, in fibroblasts, 90% of the glucosylceramide are derived from recycling of sphingolipid bases and only 10% are synthesized de novo.

Lysosomes are present in all cell types. They are membrane-bound organelles containing about 25 lysosomal membrane proteins[2] as well as vacuolar ATPase (v-ATPase) proton pump. They also have 50 known hydrolytic enzymes[2] that participate in the turnover of macromolecules with their associated accessory proteins that also participate in the turnover of the macromolecules. They include glycosidase, lipase, nuclease, phosphatase, protease, and sulfatase that are active at acidic pH and lysosomal pH in the 4.5 to 5 range. Similar to the secretory proteins, lysosomal hydrolases are glycoproteins. Lysosomal enzymes are synthesized in the rough endoplasmic reticulum (RER; Figure 1) following which they undergo a series of posttranslational modifications involving protein and carbohydrate recognition signals, allowing them to reach their final destination in the lysosome. The hydrophobic amino-terminal signal peptide on the nascent protein directs their transport into the lumen of the RER, where they undergo glycosylation of selected asparagine residues. The signal peptide is then cleaved by the removal of 3 glucose residues and 1 mannose residue. The hydrolases are then transported to the Golgi apparatus and acquire a terminal mannose-6-phosphate on the oligosaccharide side of hydrolase via 6-phospho-*N*-acetylglucosamine transferase, which later serves as a recognition marker. Hydrolases that contain terminal mannose-6-phosphate are recognized and bound by shuttled mannose 6-phosphate in the Golgi apparatus and are transferred to the mannose-6-phosphate receptor (MPR), that is, a lysosomal membrane protein. Upon their arrival into the lysosome, the hydrolases are dissociated from the shuttle receptor. The transporter then shuttles back to the Golgi to pick up another hydrolase to transfer it to the lysosome. Deficiency of the Golgi enzyme *N*-acetylglucosamine 1-phosphotransferase results in the deficiency of multiple lysosomal enzymes. The formation of this Golgi enzyme is coded by 3 different genes, and mutation of these genes leads to multiple enzyme deficiencies, that is, mucolipidosis (ML)-II (ML-II) and ML-III. A few lysosomal molecules including hydrolases and activated proteins are transmitted via sortilin that is a lysosomal membrane protein.[3] It is noteworthy that all lysosomal hydrolases are soluble proteins, with the exception of glucocerebrosidase and acid phosphatase. These lysosomal membrane proteins reach the lysosome independent of the hydrolase pathway.

Lysosomal membrane proteins are classified into several different groups of small metabolite exporter proteins including cystinosin, sialin, and cobalamin transporters. These exporter proteins participate in the efflux of the degradation products such as cysteine, free sialic acid, and cobalamin from the

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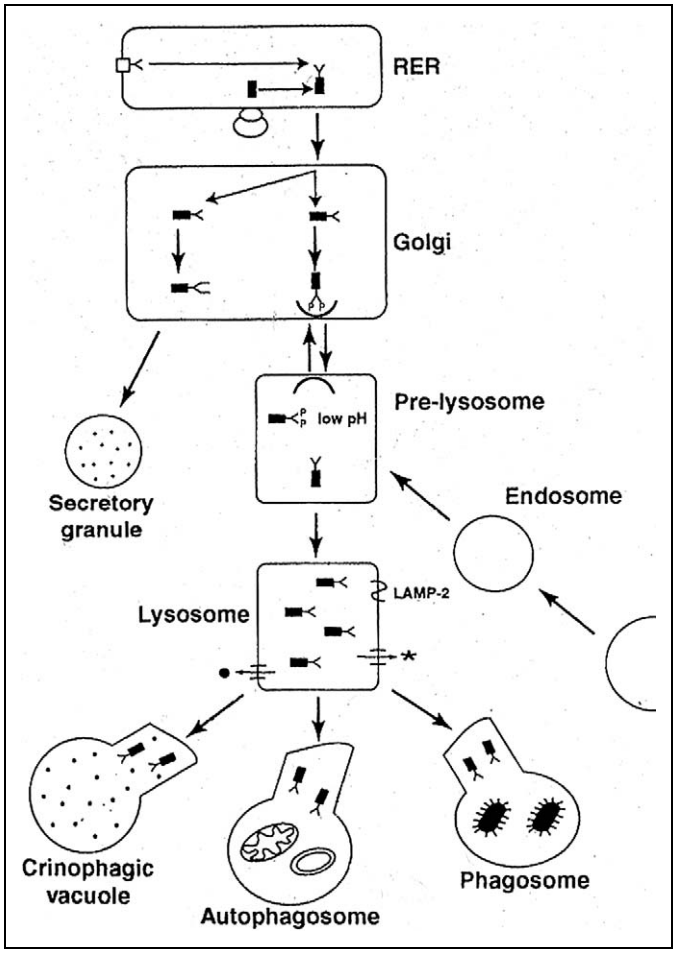

Figure 1. Schematic illustration of synthesis and targeting of lysosomal hydrolases to lysosomes, the mechanisms for transferring substrates to lysosomes for degradation, and the efflux of cystinosin and sialin. Nascent lysosomal hydrolases, secretory, and membrane proteins are glycosylated in the rough endoplasmic reticulum (RER) by transference of preformed oligosaccharides from dolichol-P-P-oligosaccharides. These glycosylated glycoproteins are translocated to the Golgi apparatus, where secretory and membrane glycoproteins are further modified and oligosaccharides of lysosomal hydrolases are phosphorylated (P). All but 2 lysosomal enzymes bind to the M-P receptors and are transported to a prelysosome compartment, where the enzymes are released and the receptor shuttles back to the Golgi apparatus. In the primary lysosome, the prohydrolases are cleaved. The secondary lysosome is formed by fusion of a phagosome with the primary lysosome via lysosomal-associated membrane protein 2 (LAMP-2). The catabolic products exit via transporter proteins such as sialin and cysteine (*).

lysosome to the cytosol hybrid enzyme/transporter protein, a defect in 4 different genetic transmembrane enzymes that are defective in mucopolysaccharidosis (MPS)-III (MPS-III) and result in the storage of heparan sulfate. Defect in ion transporter proteins, such as proton/chloride transporter *ClC-7* and *Ostm1*, results in osteopetrosis with neuronal storage and a transient receptor potential (*TRP*) defective mucolipin 1 (ML-1) protein, which is an ion channel and leads to ML-IV. Niemann-Pick C 1 (NPC-1) is a membrane protein that has a role in cholesterol transport from late endosome and lysosome into the cytosol; the lysosomal-associated membrane proteins (LAMPs), LAMP-1 and LAMP-2, are the best known. The lysosomal-associated membrane protein 2 is involved in the fusion of phagosomes with the lysosomes. Mutation of the gene that codes the LAMP-2 results in an autophagy disorder caused by defective fusion of the autophagosomes and the lysosomes.[4]

Lysosomal storage diseases are a group of inherited and acquired disorders. To date, 51 genetically determined lysosomal storage diseases have been described. Since there can be different mutations of the same gene, they result in different clinical manifestations and are classified as infantile and adult types (Table 1). The acquired disorders are the result of inhibition of α-mannosidase II by ingestion of plant materials of *Astragalus lentginous*, *Oxiftropis serica*, *Swainsona canescens*, and *Ipomoea carena* as well as treatment with certain drugs, such as amphophilic cationic drugs, amiodarone and chloroquine, which induce phospholipidosis and MPS. There are 9 different inherited and 2 acquired mechanisms that are known to cause lysosomal storage diseases (Table 2).

Most lysosomal storage diseases affect different cell types, tissues, and organs. The brain lesions are particularly prevalent, which comprises two-thirds of all lysosomal diseases.[5] The amount of substrate to be catabolized is influenced by the composition of the cell membrane, the rate of membrane recycling, the rate of cellular catabolism, the life span of the cells, the phagocytic ability of the affected cells, and the nature of phagocytosed substrates. The catabolism of macromolecules in the lysosomes can be regarded as a linear sequence of reactions, in which the product of one reaction serves as substrate for the next. Therefore, the normal and abnormal concentration of any substrate is determined by the influx rate (Vi) of the substrate and the kinetic parameters of the degrading enzymes. They include Michaelis constant (Vm), which is the enzyme–substrate affinity, that is, the binding ability and maximal velocity of the reaction (Vmax), which is obtained when all the enzyme molecules are saturated with substrate.

The majority of these diseases are due to the deficient activity of the lysosomal enzymes caused by mutations of genes coding specific hydrolases. However, the complexity of lysosomal storage disease is seen in mutations of nonenzymatic proteins.[5] The clinical heterogeneity of any one of the lysosomal enzymes is caused by the multiplicity of mutant alleles of a given gene, which leads to difference in the properties of residual enzymes or absence of enzymes.

Clinically, early and severe manifestations are due to the absence of enzyme activity and early death. In cases of low enzyme activity, there is late presentation and mild to moderate manifestations. Normally, most lysosomal hydrolases are present in sufficiently high amount that their respective substrate does not accumulate. Accumulation of undegraded substrate occurs only when the residual enzyme activity is less than the critical threshold of 10% to 15% of the normal enzyme activity. Usually, the residual enzyme activity results in juvenile or adult onset of the disease, and absence of enzyme activity is manifested as severe infantile onset. The third mechanism is due to mutation that results in a defective or absence of the key enzyme, 6-phospho-*N*-acetyl-glucosamine transferase, leading

Table 1. Lysosomal Storage Diseases.

Disease	Deficient Hydrolases	Primary Storage Products	Major Organs Involved	Corresponding Disease in Animals
MPS				
MPS-I Hurler, Scheie syndrome	α-L-Iduronidase	Dermatan sulfate, heparan sulfate	CNS, connective tissue, heart, skeleton, cornea	DSH cats, Plott hounds
MPS-II Hunter syndrome	Iduronate sulfatase	Dermatan sulfate, heparan sulfate	CNS, connective tissue, heart, skeleton	*Labrador Retriever* dogs
MPS-III Sanfilippo syndrome				
Subtype A	Sulfaminidase	Heparan sulfate	CNS	Wire-haired Dachshund, New Zealand Huntaway dogs
Subtype B	α-*N*-Acetylglucosaminidase	Heparan sulfate	CNS	Emus, Schipperke dogs
Subtype C	Acetyl Co A α-glucosaminide acetyltransferase	Heparan sulfate	CNS	
Subtype D	*N*-acetylglucosamine 6-sulfatase	Heparan sulfate	CNS	Nubian goats
MPS-IV Morquio syndrome				
Type A	Galactose 6-sulfatase	Chondroitin-4 sulfate, keratan sulfate	Cartilage, skeleton, cornea, heart	
Type B	β-Galactosidase	Keratan sulfate	Cartilage, bone, cornea	
MPS-VI Maroteaux-Lamy syndrome	Arylsulfatase β-*N*-acetylgalactosamine 4-sulfatase	Dermatan sulfate	Skeleton, cornea, heart	DSH Siamese Cats, rats, schnauzers, miniature pinschers, and Welsh Corgi dogs
MPS-VII Sly syndrome	β-Glucuronidase	Dermatan sulfate, heparan sulfate, chondroitin 4-8-sulfate	CNS, connective tissue, skeleton, heart	Dogs, DSH Cats, Gus mice
MPS-IX hyaluronidase deficiency	Hyaluronidase	Hyaluronan	Periarticular soft tissue	
Sphingolipidoses (lipid storage diseases)				
Glucocerebrosidosis (Gaucher disease)				
Infantile type 2	β-Glucocerebrosidase	Glucosylceramide	CNS, spleen, liver, bone marrow	Silky Terrier dogs, Sheep, pigs
Juvenile type 3			CNS, spleen, liver, bone marrow	
Adult type 1			Spleen, liver, bone marrow	
Fabry disease	α-Galactosidase	Trihexosylceramide	Blood vessels of skin, kidney, and brain	
Schindler disease (type I)	α-*N*-acetylgalactosaminidase	Sialylated and asialopeptides and oligosaccharides	CNS, PNS	
Type II	α-*N*-acetylgalactosaminidase	Sialylated and asialopeptides and oligosaccharides	CNS, PNS	
Metachromatic leukodystrophy				
Late infantile form	Arylsulfatase A	Galactosylsulfatide	CNS, liver, kidney, gallbladder	Hawaiian geese
Late-onset form	Arylsulfatase A			
Multiple sulfatase deficiency	At least 7 lysosomal sulfatases and a microsomal sulfatase		CNS, visceral organs, and skeleton	
Niemann-Pick disease				
Type A	Sphingomyelinase	Sphingomyelin	CNS, liver, spleen, bone marrow	Poodles, boxers, Balinese, DSH, and Siamese cats
Type B				

(continued)

Table 1. (continued)

Disease	Deficient Hydrolases	Primary Storage Products	Major Organs Involved	Corresponding Disease in Animals
Type C (NPC-1 and NPC-2)	Proteins required for lipid transport through late endosome	Unesterified cholesterol and spingolipids	CNS, liver, spleen	Flight model mice, DSH cats, boxers
G_{M1}-gangliosidosis	β-galactosidase	G_{M1}-ganglioside, oligosaccharides, keratan sulfate	CNS, skeleton, viscera	DSH, Korat and Siamese cats, English Springer Spaniels, Portuguese water dogs, Shebas, Alaskan Huskies, Beagle dogs, Friesian cattle, Coopworth-Romney, Suffolk cross-bred sheep, and American black bears
G_{M2}-gangliosidosis				
Tay-Sachs disease, A variant	β-hexosaminidase A	G_{M2}-ganglioside	CNS	Muntjac deer, flamingos, Yorkshire pigs, Jacob sheep, raccoons
Sandhoff disease	β-hexosaminidases A and B	G_{M2}-ganglioside, oligosaccharides	CNS	DSH, European Burmese, Japanese domestic and Korat cats, German Short-hair Pointers, Golden retriever dogs, and Yorkshire pigs
AB variant	Deficiency of G_{M2} -activator protein	G_{M2}-ganglioside	CNS	Japanese Spaniel, DSH, emu
Galactosialidosis	Protective protein/ cathepsin A, resulting in deficiency of β-galactosidase and α-neuraminidase	Glycolipids and oligosaccharides	CNS, spleen, liver, skeleton	Suffolk cross-breed sheep, Schipperke
Globoid cell leukodystrophy (Krabbe disease)	Galactosylcerebroside, β-galactosidase	Galactosylsphingosine	CNS	Cairn and West Highland terriers, poodles, beagles, blue tick hound dogs, Pomeranians, Irish Setters, Twitcher mice, Polled Dorset sheep, DSH Cats, rhesus monkeys
Farber granulomatosis	Ceramidase	Ceramide	Subcutaneous nodules, joints, larynx, liver, lung, heart	
Wolman disease	Acid lipase/cholesterol esterase	Triglycerides, cholesteryl esters	Liver, spleen, adrenal	Sheel Parakeets, Donryu rats, Fox terriers
Disorders of glycoprotein degradation				
Aspartylglucosaminuria	Aspartylglucosaminidase	Fragments of glycoprotein, aspartyl-2-deoxyl-2-acetamide glycosylamine, *N*-linked oligosaccharides	CNS, connective tissue, bone marrow	
α-Mannosidosis	α-Mannosidase	*N*-linked oligosaccharides	CNS, skeleton, liver, spleen	Angus, Shorthorn cattles, Simmentals, Galloways, Murray Grey cattle, DSH, DLH, and Persian cats
β-Mannosidosis	β-mannosidase	*N*-linked oligosaccharides	CNS, skeleton, liver, spleen	Anglo-Nubian goats, Saler cattle
Fucosidosis	α-fucosidase	Oligosaccharides and Glycolipids	CNS, spleen, liver	English Springer Spaniels, English Pointer dogs
Sialidosis (mucolipidosis-I)	Neuraminidase	Fragments of glycoprotein	CNS, spleen, liver, skeleton	DSH cats
Neuronal ceroid lipofuscinosis (NCL)				
Infantile NCL (CLN-1)	Palmitoyl protein thioesterase	Protein, saposins A and D	CNS, Heart, endothelial Cells, retina	Swedish sheep, ferrets

(continued)

Table 1. (continued)

Disease	Deficient Hydrolases	Primary Storage Products	Major Organs Involved	Corresponding Disease in Animals
Congenital NCL (CLN-10)	Cathepsin D (CTSD)	Protein	CNS, Liver	American Bulldogs, White Swedish Landrace sheep
Classic late infantile NCL (CLN-2)	Pepstatin-sensitive protease, tripeptydyl-peptidase	Mitochondrial subunit C of ATPase synthase	CNS, retina	Australian cattle dogs, blue heelers, and border collies, Dachshund dogs
Late infantile (Indian Variant) NCL (CLN-6)	Endoplasmic reticulum transmembrane protein	Mitochondrial subunit C of ATPase synthase	CNS, heart, endothelial cells	Merino, Rambouillet, South Hampshire sheep, English Setter and blue heeler dogs, Devon cattle, DSH cats
Late infantile (Turkish variant) NCL (CLN-7)	Protein	Mitochondrial subunit C of ATPase synthase	CNS	
Late infantile (Finnish variant) NCL (CLN-5)	Transmembrane protein	Mitochondrial subunit C of ATPase synthase	CNS, heart, endothelial cells	Borderdale sheep, Devon cattle
Juvenile NCL (CLN-3)	(Battenin) Lysosomal transmembrane protein	Mitochondrial subunit C of ATPase synthase	CNS, heart, endothelial cells, retina	Chihuahua, Cocker Spaniel, Dachshund, Dalmatian, English Setter, Saluki, Tibetian terrier, Yugoslavian shepherd dogs, Siamese cat, South Hampshire and Swedish sheep.
Adult NCL (CLN-4)	Unknown	Mitochondrial subunit C of ATPase synthase	CNS, heart, endothelial cells	American Staffordshire terriers
Progressive epilepsy with mental retardation, Northern epilepsy (CLN-8)	Transmembrane protein	Mitochondrial subunit C of ATPase synthase	CNS	English Setter dogs
Juvenile variant (CLN-9)	Unknown		CNS	
Glycogen storage disease				
Pompe disease, type II				
Early onset	α-Glucosidase (acid maltase)	Glycogen	CNS, muscle, heart	Brahman and shorthorn cattle, Lapland dog, cat, Corriedale sheep, and Japanese Quail
Late onset	α-Glucosidase (acid maltase)	Glycogen	CNS, muscle, heart	
Disorders of protein degradation				
Pycnodysostosin	Cathepsin K	Collagen	Osteochondrodysplasia	
Experimental dilated cardiomyopathy	Cathepsin L	Sarcomeric protein	Heart	Mice L^{-1}
Papillon-Lefèvre syndrome	Cathepsin C	Proteins	Periodontal disease and palmoplantar keratosis	Mice^{-1}-B^{-1}
Neuronal loss and brain atrophy	Cathepsins B and L	Proteins	CNS	
Abnormal lysosomal membrane transport				
Mucolipidosis-II (I-cell disease)	*N*-acetylglucosaminyl-phosphotransferase resulting in multiple enzyme deficiencies	Mucopolysaccharides, lipids, glycoproteins	CNS, connective tissue, skeleton, heart, kidney	DSH cats
Mucolipidosis-III (pseudo-Hurler polydystrophy)	*N*-acetylglucosaminyl-phosphotransferase resulting in multiple enzyme deficiencies	Mucopolysaccharides, lipids, glycoproteins	Joint and connective tissue predominantly	
Mucolipidosis-IV	TRPML-1	Lipids	CNS, connective tissue	
Danon disease	Deficiency of LAMP-2	Glycogen	CNS, muscle, heart	
Infantile osteopetrosis and neuronal	OSTM1 Chloride Channel 7	Glycoproteins and glycolipids	CNS, skeleton	

(continued)

Table 1. (continued)

Disease	Deficient Hydrolases	Primary Storage Products	Major Organs Involved	Corresponding Disease in Animals
Disorders of lysosomal efflux				
Cystinosis	Cysteine efflux mediator	Cysteine	Kidney	
Salla disease	Sialic acid efflux mediator	Free sialic acid	CNS	
Infantile dialiuria	Sialic acid efflux mediator	Free sialic acid	CNS, kidney, liver	
Other				
Acid phosphatases	Acid phosphatase		CNS, skeleton	

Abbreviations: CNS, central nervous system; CLN, neuronal ceroid lipofuscinoses; DSH, domestic short-haired cats; DLH, domestic long-haired cats; FM, flight model; LAMP-2, lysosomal-associated membrane protein 2; MPS, mucopolysaccharidoses; NPC, Niemann-Pick C; PNS, peripheral nervous system; TRPML-1, transmembrane protein mucolipin 1.

Table 2. Classification of Mechanism of Lysosomal Storage Disorders

Mechanisms	Examples
Disorders in which no immunologically detectable enzyme is synthesized; include conditions with grossly abnormal structural genes	Infantile forms of glycoprotein, glycolipid, glycogen, or liquid storage diseases and mucopolysaccharides
Disorders in which a catalytically inactive polypeptide is synthesized; the mutation may also affect the stability or transport of the polypeptide	Juvenile or adult forms of glycoproteins, glycolipids, glycogen, or lipid storage diseases and mucopolysaccharides
Disorders in which a catalytically active enzyme is synthesized, but the enzyme is not segregated into lysosomes	I-cell disease, pseudo-Hurler polydystrophy
Disorders in which a catalytically active enzyme is synthesized; however, the enzyme is unstable in prelysosomal or lysosomal compartments	Galactosialidosis
Disorders in which activator proteins (saposins) of liquid-degrading hydrolases are missing	AB variant of GM_2-gangliosidosis, variants of Gaucher and Farber diseases, juvenile variant of meta-chromatic leukodystrophy
Disorders in which the structural gene of the hydrolase is normal, but there is a mutation of the genes that codes for posttranslational modification of the hydrolase	Multiple sulfatase deficiencies
Disorders due to abnormal transport to the lysosomes	Mucolipidosis-IV, NPC-1, and NPC-2. Infantile osteopetrosis with neuronal storage
Disorder due to lack of fusion between endosome and lysosome due to mutation of the gene that codes for lysosomal membrane protein 2 (LAMP-2)	Danon disease
Disorders due to a decrease in transport of degradation end products (ie, free cysteine) out of the lysosomes	Salla disease, infantile sialidosis, and cystinosis
Disorders due to oversupply of substrate	Some cases of chronic myelocytic leukemia, sickle-cell anemia, thalassemia that are associated with Gaucher-like cells, that is, sea-blue histiocytes
Disorders in which deficiencies in lysosomal enzyme activities result from intoxication with neutral or synthetic inhibitors of lysosomal enzymes	Cationic amphophilic drugs (ie, amiodarone, chloroquine, swainsonine)

Abbreviation: NPC, Niemann-Pick C.

to absence of the recognition marker on the lysosomal enzyme and, as a consequence, the enzyme is not transported to the lysosome but instead is secreted from the cells. In the absence of functional protective protein/cathepsin A, rapid degradation of β-galactosidase and neuraminidase ensues, thus resulting in deficient activity of both enzymes. Lysosomal degradation of sphingolipid by acid hydrolase requires activator proteins (saposins) that are small nonenzymatic cofactors assisting the enzymes in attacking sphingolipids. Mutations of any one of these cofactors result in lysosomal storage diseases. Mutations of posttranslational enzyme that is located in the RER and activates sulfatases result in multiple sulfatase deficiency. Mutations of LAMP-2 lead to the accumulation of phagosomes with storage material. Mutations of transporter molecules such as NPC-1, NPC-2, and (ML-1) also result in lysosomal storage. In general, it is assumed that the breakdown products in the lysosome, such as monosaccharides, amino acids, and nucleotides, diffuse across the lysosomal membrane to the cytosol for reuse. However, mutations of lysosomal transmembrane proteins cystinosin and sialin result in the storage of cysteine and free sialic acid. Mutations of chloride channel 7 result in osteopetrosis and neuronal storage. However, the cause for neuronal storage is still unknown. Ingestion of plants like *Astralagus lentginosus*, *Oxiftrophis serica*, *Swanisona canescens*, *Ipomea carnea*, and *Sida carpinifolia* result in acquired lyosomal storage disease.[6] This is due to the inhibition of α-mannosidase II with swainsonine and 2 glycosidase inhibitors, calystegine B and calystegine C_1, during posttranslational modification of the lysosomal hydrolase. Treatment with

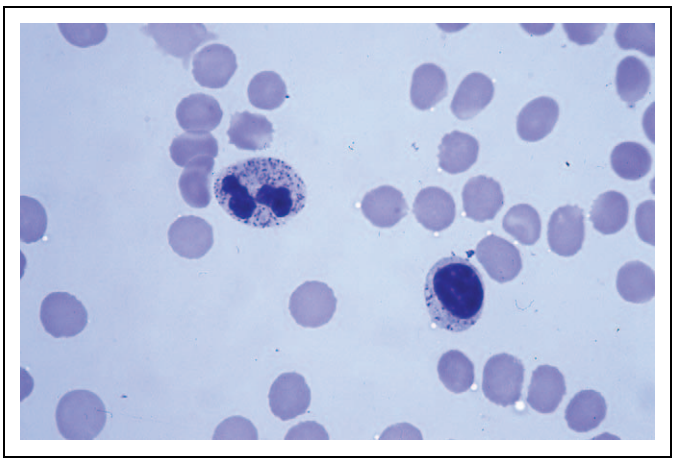

Figure 2. Blood smear of a patient with multiple sulfatase deficiency showing a lymphocyte and neutrophil stained with Wright-Giemsa. The cytoplasm of both cells contains metachromatic granules.

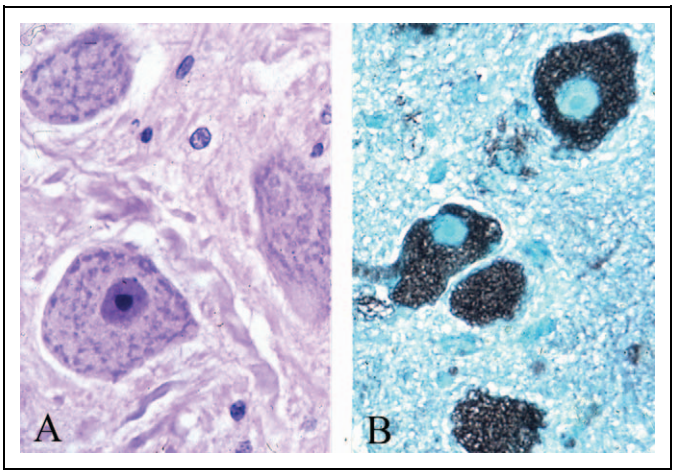

Figure 3. Photomicrographs of a cow cerebrum with α-mannosidosis illustrating (A) enlarged neurons with vacuolated cytoplasm, hematoxylin and eosin (H&E). B, Staining with Con A demonstrating the storage of oligosaccharides with terminal α-mannosyl residues.

cationic amphophilic drugs,[7] such as amiodarone, chloroquine and tilorone,[8] results in lysosomal storage of phospholipids. The primary mechanism of these drugs that leads to phospholipidosis is an inhibition of lysosomal phospholipase as well as an increase in the pH within the lysosomes. In addition, drugs like tilorone cause storage of sulfated glycosaminoglycans. In several hematologic disorders, oversupply of substrates for degradation by macrophages leads to lysosomal storage in macrophages.

Generally, 2 types of changes are observed in lysosomal storage diseases. The primary changes include the direct consequence of abnormal accumulation of substrates or catabolites in the lysosomes, leading to severe impairment of cellular structures and functions and abnormal extracellular matrix. They include alterations of signaling pathways, of intracellular Ca^{2+} homeostasis, of intracellular trafficking, of lipid biosynthesis, dysmyelinogenesis, formations of ectopic dendrites, retarded bone formation, and cloudy corneas. In some diseases, abnormal accumulation of galactosylsphingosine, that is psychosine, leads to apoptosis.[9] The secondary changes are due to disrupted recycling and are manifested in abnormal or shortage of cellular and extracellular products. In the brain, there is a compromised recycling of neurotransmitter receptors, altered signaling,[10] and dysmylinogenesis.[11] For example, disarray of the collagen fibers leading to cloudy cornea was demonstrated in MPS-I and -VI and retarded bone formation,[12] as was reported in G_{M1}-gangliosidosis and MPS.

The clinical manifestation of some lysosomal storage diseases is very similar, and they are often similar to other developmental and neurological disorders. Therefore, the diagnosis of a specific lysosomal storage disease requires a combination of clinical, morphological, biochemical, and molecular biological techniques.[13] The preliminary diagnosis of most of the lysosomal storage diseases could be made by microscopic examination of the blood smear (Figure 2). If there is an indication of storage disease in paraffin and frozen sections, lectin histochemistry is a useful tool.[14] Electron microscopy of skin biopsies is a useful diagnostic tool for the diagnosis of lysosomal storage diseases, since the skin contains all the body cell types and by observing the morphological features of the storage material that can be identified. Diagnosis of MPS and diseases with interrupted catabolism of oligosaccharides may be determined by urine analysis. Different diagnostic methods such as quick spot test, electrophoresis of urinary glycosaminoglycans, and fluorophore-labeled carbohydrate polyacrylamide gel electrophoresis are used.[13] Specific diagnosis of the majority of lysosomal storage diseases can be achieved by means of assay of the activity of lysosomal hydrolases in white blood cells, cultured fibroblasts, serum, and molecular biology. Postmortem diagnosis is established by both biochemical and morphological examinations. The former includes enzyme assays and biochemical characterization of the storage material, both of which can be done on fresh or frozen tissues. Enlargement and vacuolization of the affected cells (Figure 3A) may be indicators of lysosomal storage disease. Positive staining of the vacuolar membrane with LAMP-2 antibodies will identify the vacuoles as lysosomes. Staining of paraffin and frozen sections with *Periodic acid–Schiff* (PAS), PAS-diastase, Alcian blue, or colloidal iron, both with and without treatment with hyaluronidase and various lectins (Figure 3B), is useful in the identification of the glycoconjugate residues in oligosaccharides and glycolipids, respectively. Sudan black (Figure 4), oil red O, and luxol fast blue (LFB; Figure 5) are helpful in the characterization of lipid storage. Storage of autofluorescent material is associated with neuronal ceroid lipofuscinosis (NCL; Figure 6) and other storage diseases. Electron microscopic examination of the affected cells provides a cost-effective method for the diagnosis of lysosomal storage diseases.[15] It is noteworthy that in some variants of ceroid lipofuscinosis, the combination of clinical manifestation and the ultrastructure is still the only tool for characterizing the disease. The presence of secondary lysosomes in affected cells and the ultrastructure of the storage material may indicate the nature of the storage material. Coarse lamellated membrane structures are observed in sphingomyelin and gangliosides (Figure 7A and B); twisted microtubules or cleft-like structures are observed in storage of cerebrosides (Figure 8). In storage of mucopolysaccharides, the lysosomes contain fine

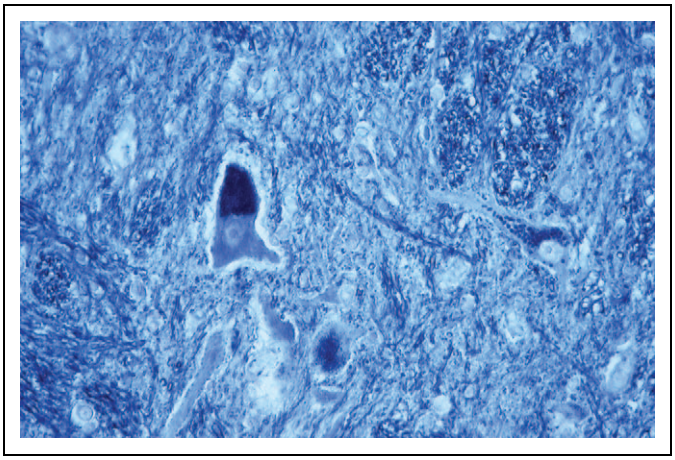

Figure 4. Photomicrograph of frozen section of spinal cord of a 1½-year-old Australian Blue Heeler dog with neuronal ceroid lipofuscinosis in spinal cord stained with Sudan black revealing the storage of ceroid lipofuscin within the neurons.

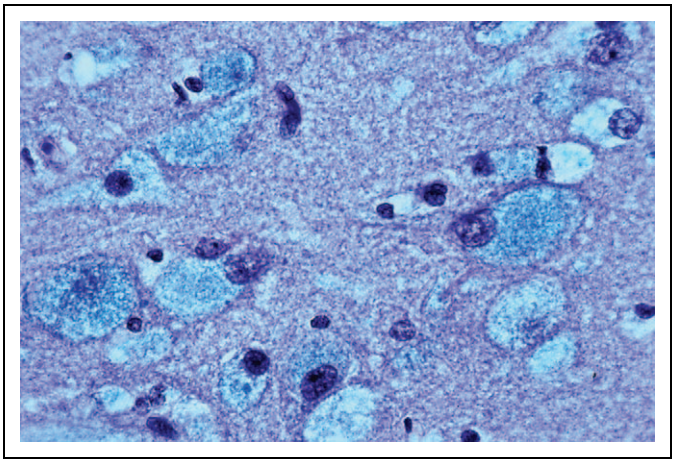

Figure 5. Paraffin section of the brain of a 7-month-old English Springer Spaniel dog stained with Luxol Fast Blue demonstrating the storage of G_{M1}-gangliosides.

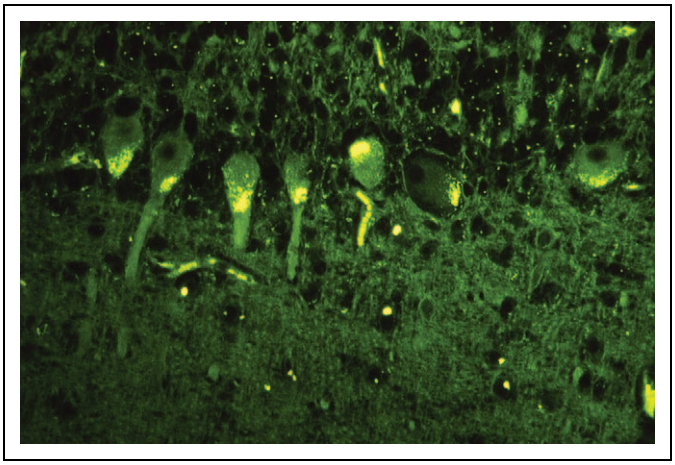

Figure 6. Photomicrograph of the cerebellum of New Zealand sheep with neuronal ceroid lipofuscinosis revealing the presence of autofluorescent neurons.

fibrillar material together with lamellated membrane structure (Figure 9). Very fine fibrillar material or electron-lucent lysosomes are observed in storage of oligosaccharides (Figure 7C) or free sialic acid. Neutral lipids and cholesterol clefts are seen in acid lipase deficiency; banana- and crystal-shaped lysosomes are characteristic of ceramidase deficiency. Granular osmiophilic deposits (GRADs) are characteristic of infantile NCL, and curvilinear structures (Figure 10) and fingerprints are seen in several different variants of NCL. In addition, spheroids are often seen in axons and occasionally in the cytoplasm of neurons (Figure 7D).

Mucopolysaccharidosis

Mucopolysaccharidosis is a group of lysosomal storage diseases, which results from the deficiency of specific lysosomal enzymes involved in the degradation of mucopolysaccharides, that is, glycosaminoglycans that are abundant in the extracellular matrix. Chondroitin, dermatan, heparin, and keratan sulfates are the major constituents of the glycosaminoglycan portions of proteoglycans (PGs). The majority of the PGs are present in the extracellular matrix interacting with or binding to collagen, elastin, fibronectin, laminin, and plasma membrane. The hyaluranan is mostly present in soft connective tissue. The inheritance of all these diseases with the exception of MPS-II is autosomal recessive. Mucopolysaccharidosis-II is an X-linked disorder. In all these diseases, except in MPS-IIIC, the deficient enzymes are hydrolases. In these diseases, there is multiple tissue and organ involvement, in particular those that are rich in mesenchymal cells such as the skeletal tissue and cornea. In addition to the primary changes, that is, lysosomal storage, secondary changes such as skeletal development and cloudy cornea are also observed.[10] It has been reported that cloudy cornea as seen in MPS-I and MPS-VI is due to alteration in the corneal stroma (Figure 11).[16] In MPS-IX, the changes are periarticular. In general, the Berry spot test that is done on urine samples is often very useful for early diagnosis of MPS, but enzyme analysis is the gold standard to confirm the diagnosis. The presence of metachromatic granules in leukocytes is indicative of MPS.

Mucopolysaccharidosis-I

Deficient activity of α-L-iduronidase primarily results in the storage of dermatan and heparan sulfate. Depending on the mutation, it has a wide range of clinical manifestations. Hurler syndrome is the most severe; today, Hurler-Scheie and Scheie forms are considered the "attenuated forms" of MPS-I. Mucopolysaccharidosis is reported in domestic short-hair (DSH) cats and Plott hounds. The severe form occurs in both species, and they are manifested by severe neurological signs, facial and skeletal dysmorphia, corneal clouding, and cardiac valve insufficiency. Different cell types, including neural, mesenchymal, and epithelial cells, are affected. They become enlarged and contain lysosomes that are stainable with PAS, alcian blue, and colloidal iron and are metachromatic when stained with toluidine blue. Affected neurons containing stored G_{M1}, G_{M2}, and G_{M3} are stainable with Luxol fast blue and Sudan black.

Figure 7. Electron micrographs of the cerebellum from a 5½-month-old Portuguese water dog. A, Low magnification illustrating on the left neuronal cytoplasm packed with enlarged secondary lysosomes, at the center is a section through an endothelial cell, and on the right is an axon. B, High magnification reveals lysosomes packed with gangliosides. C, Close-up view of the endothelial cell that has enlarged electron-lucent lysosomes containing oligosaccharides. D, On the right, there is high magnification of an axon packed with spheroids.

Mucopolysaccharidosis-II

Mucopolysaccharidosis-II or Hunter syndrome is transmitted as an X-linked recessive disorder that causes the deficiency of iduronate sulfatase activity. This results in primary storage of dermatan sulfate and heparan sulfate. An adult form has been described in Labrador Retriever dogs that are presented with course facial features, visual impairment, progressively neurological, and osteopenia. Similar to MPS-I, neuronal, mesenchymal, and epithelial cells are affected. They are enlarged, vacuolated, and stained with PAS and Alcian blue.

Mucopolysaccharidosis-III

Mucopolysaccharidosis-III or Sanfilippo disease is a group of diseases that are caused by mutations of 4 different enzymes but have a common primary storage material, that is, heparan

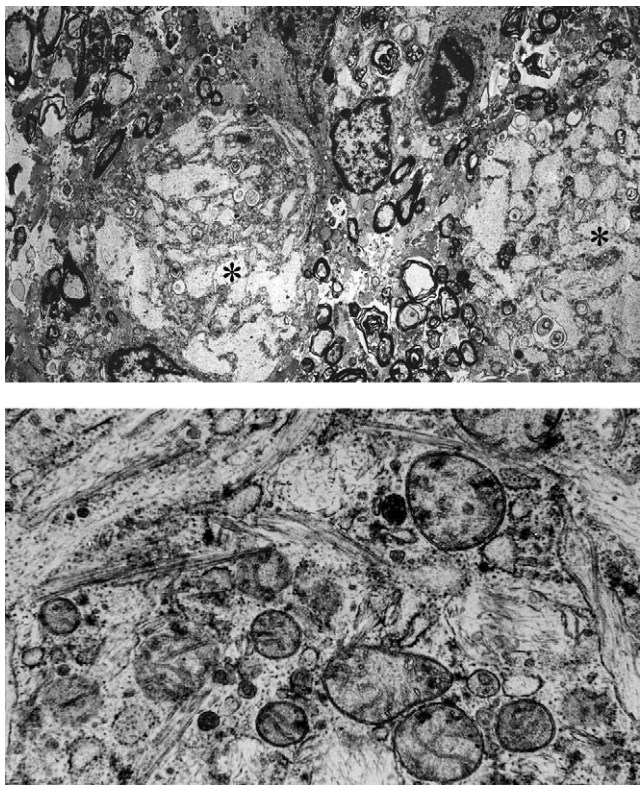

Figure 8. Low (A) and high magnification (B) of a brain from a rhesus monkey with Krabbe disease demonstrating globoid cells (*) that have cleft-like storage material.

Figure 9. Electron micrograph of glomerular mesangial cell of a Siamese cat with mucopolysaccharidosis (MPS)-IV revealing secondary lysosomes that contains a mixture of lamellated membrane structures and fine fibrillar material.

sulfate. Unlike the other components of glycosaminoglycans, which are the major components of the extracellular matrix, heparin sulfate is a component of transmembrane syndecan.

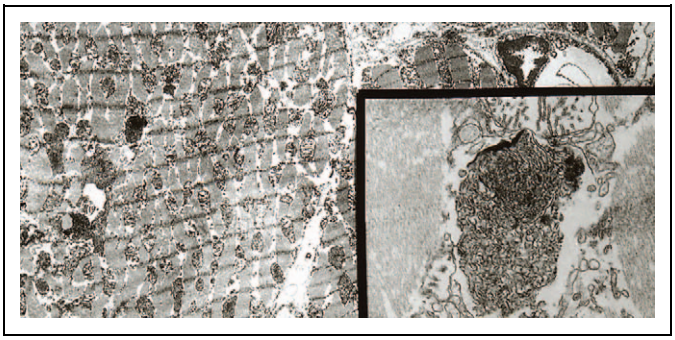

Figure 10. Low-magnification electron micrograph of myocardial cells from a 2-year-old Australian Blue Heeler dog with neuronal lipofuscinosis that contain secondary lysososmes. Inset: Higher magnification of a lysosome that is packed with curvilinear material.

As a result, unlike other MPS, neurological signs are the major clinical manifestation. However, it is noteworthy that in a few cases of different types of MPS-III in humans and in a colony of Nubian goats with MPS-IIID, only 1 goat had both cloudy corneas due to irregular packing of the collagen fibrils and skeletal abnormalities, indicating relatively low activity of another enzyme in the pathway, which contributes to those lesions.

Mucopolysaccharidosis-III A is caused by the deficiency of heparin *N*-sulfatase activity and has been identified in wire-haired Dachshunds and New Zealand Huntway dogs. Mucopolysaccharidosis-IIIB, caused by the deficiency of α-*N*-acetylglucosaminidase acticity, has been reported in Schipperke dogs and emus. Mucopolysaccharidosis-IIIC is due to the deficiency of acetyl Co A α-glucosaminide acetyltransferase activity. Mucopolysaccharidosis-IIID is caused by the deficiency of *N*-acetylglucoasamine 6-sulfatase activity and has been characterized in Nubian goats. Histological and biochemical studies of liver and brain of Nubian goats with MPS-IIID demonstrated lysosomal storage of glycosaminoglycan and G_{M3} gangliosides.

Mucopolysaccharidosis-IV

Mucopolysaccharidosis-IV or Morquio syndrome is a group of diseases that are caused by mutations of 2 different genes but with similar clinical manifestations and morphological changes. Mucopolysaccharidosis-IVA is caused by the deficiency of galactose 6-sulfatase activity and results in lysosomal storage of chondroitin-4-sulfate and keratan sulfate. Mucopolysaccharidosis-IVB is caused by mutations of β-galactosidase and leads to the storage of keratan sulfate. Clinically, the deficient activity of both hydrolases is manifested by corneal clouding and skeletal abnormalities.

Mucopolysaccharidosis-VI

Mucopolysaccharidosis-VI or Maroteaux-Lamy syndrome is due to the deficiency of *N*-acetylgalactosamine 4-sulfate (arylsulfatase B) activity. It results in lysosomal storage of dermatan sulfate and is clinically manifested by corneal clouding and

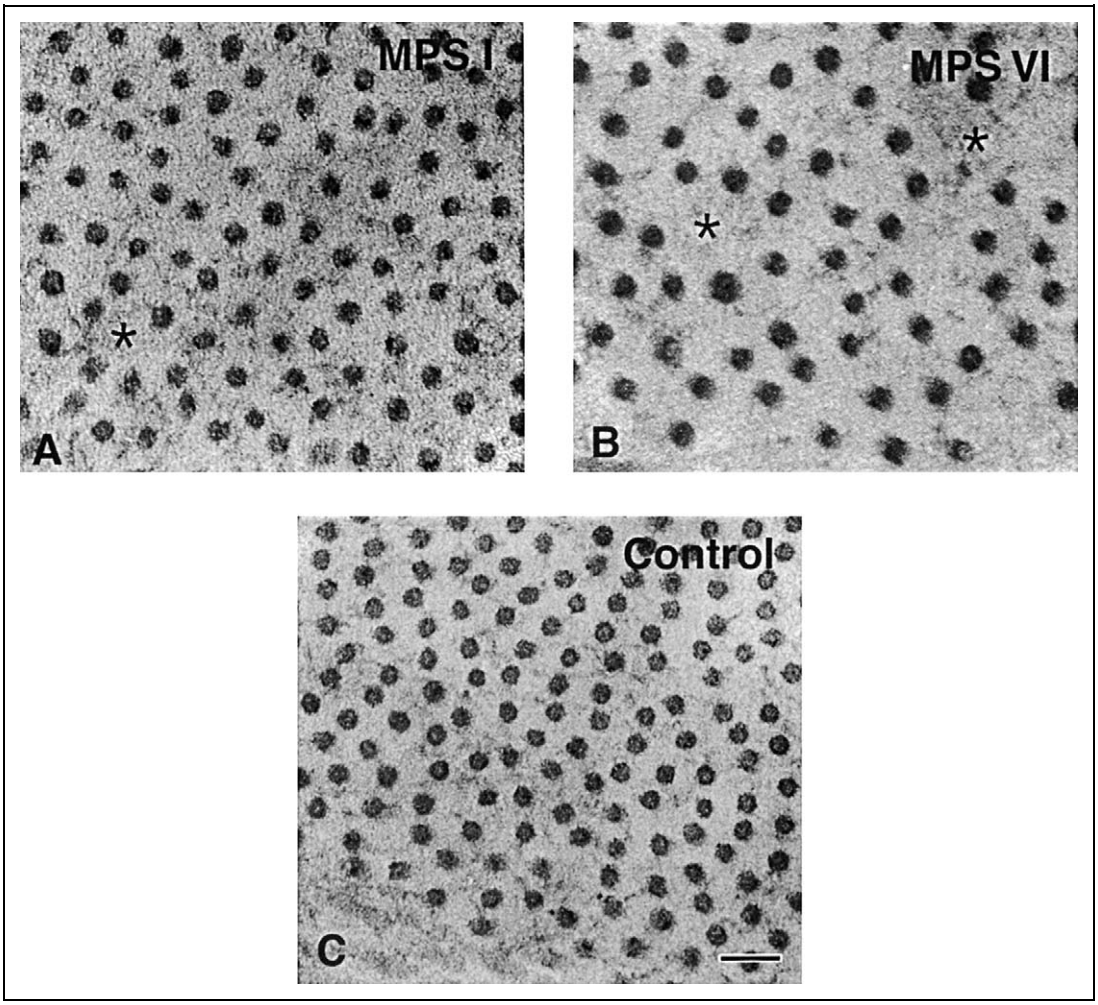

Figure 11. Electron micrograph of the corneal stroma from cats with mucopolysaccharidosis (MPS)-I (A), MPS-VI (B), both with cloudy cornea, and a normal control cat (C). There is an increase in fibril spacing and more irregular packing of fibrils (*) in the affected corneas but not in the control. The collagen fibrils from affected cats are larger and more varied in diameter size. Bar 100 nm.[16]

severe skeletal abnormalities. It has been described in DSH and Siamese cats, rats, schnauzers, miniature pinscher, and Welsh Corgi dogs.

Mucopolysaccharidosis-VII

Mucopolysaccharidosis-VII or Sly syndrome is caused by the deficiency of β-glucoronidase activity and results in lysosomal storage of dermatan sulfate, heparin sulfate, and chondroitin-4-6 sulfates. Clinical manifestations include severe skeletal abnormalities, hepatosplenomegaly, and thickening of atrioventricular heart valves. It has been described in DSH cats, mixed breed dogs, and Gus mice.

Mucopolysaccharidosis-IX

Mucopolysaccharidosis-IX is due to the deficiency of lysosomal hyaluronidase-1 activity that results in the storage of hyaluronan. Hyaluronan is a major component of extracellular matrix. Patients present with short stature, cutaneous swelling, and periarticular swelling.

Mucolipidosis-I

Mucolipidosis-I, or sialidosis, is the result of deficiency of lysosomal α-neuraminidase (sialidase) activity. Its deficiency results in lysosomal storage of sialilated oligosaccharides and sialilated glycolipids. Clinical manifestations include neurological signs, organomegaly, and skeletal abnormalities.[17] It has been described in DSH cats.

Mucolipidosis-II and Pseudo-Hurler Polydystrophy

The ML-II or I-cell disease and Pseudo-Hurler polydystrophy (ML-III) are the consequence of a primary deficiency of phospho-*N*-acetylglucosamine transferase, resulting in failure of transport-soluble hydrolases from the Golgi apparatus to the lysosome. This leads to deficiency of lysosomal hydrolases activity. Clinically, it is manifested by severe neurological

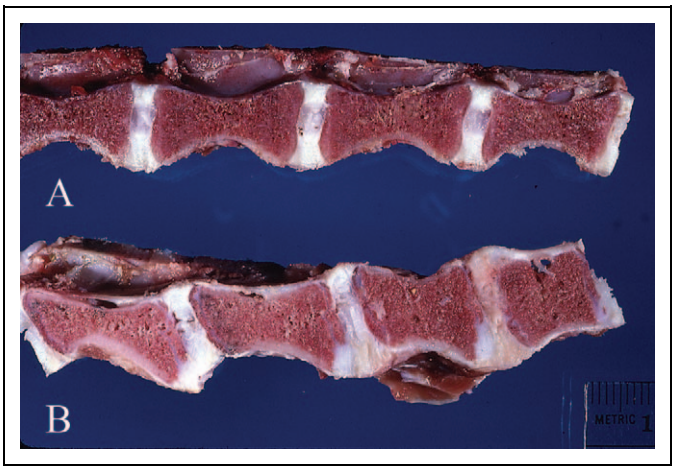

Figure 12. Photograph of lumbar vertebrae from a 3-month-old normal Portuguese water dog (A) and his brother with G_{M1}-gangliosidosis (B). In (B) the vertebrae are shorter and the intervertebral disks are irregular and wide.

signs, organomegaly, and skeletal abnormalities in ML-II and moderate to milder symptoms in ML-III.[18] I-Cell disease has been reported in DSH cats.

Mucolipidosis-IV

Mucolipidosis-IV is due to the mutation of the transmembrane protein ML-1, a transient receptor potential, also known as TRPML1.[19] It plays a major role in the fusion of autophagosomes with late endosomes/lysosomes and serves as a cation channel. Gene mutations result in defective chaperone-mediated autophagy. The clinical manifestations include psychomotor retardation, blood iron deficiency, and gastric achloridia.

Sphingolipidosis (Lipid Storage Diseases)

Sphingolipidosis refers to a group of disorders caused by deficient activity of lysosomal hydrolases that participate in the degradation of lipid that contains sphingosine as well as by mutations of the corresponding sphingolipid activator proteins (SAPs), including saposins and G_{M2} activator protein, and the protective protein/cathepsin A. The SAPs and G_{M2} activator protein are required for binding the glycolipids to their corresponding hydrolase.[20,21]

G_{M1}-Gangliosidosis

G_{M1}-gangliosidosis is caused by deficient activity of β-galactosidase, resulting in lysosomal storage of glycolipids, oligosaccharides, and glycosaminoglycans, with a nonreducing terminal β-galactoside.[22] These molecules are major constituents of cell membrane and extracellular matrix. Therefore, almost all cell types are affected, but they differ in the nature of the storage materials. Neurons contain numerous lamellated membrane structures that are often described as fingerprints or zebra bodies (Figure 7A and B), and on frozen sections they

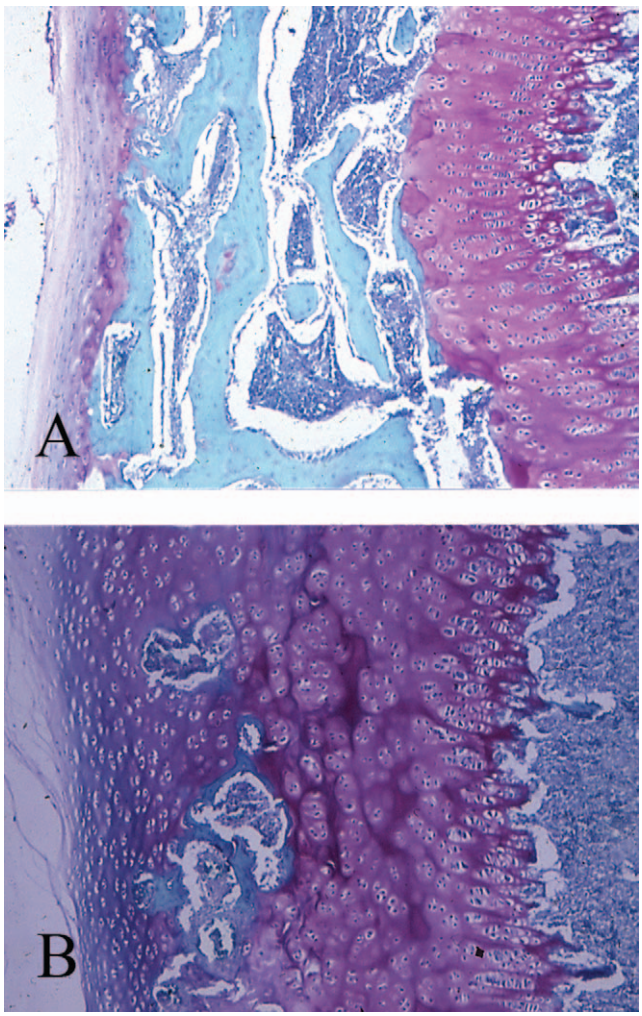

Figure 13. Longitudinal section of part of a lumbar vertebra of a 2-month-old normal Portuguese water dog and his affected sex- and age-matched brother. B, In the normal puppy (A), the ossification is advanced and the primary spongiosa are well developed. In the affected puppy (B), the ossification is retarded, the primary spongiosis is poorly developed and there is metaphysical osteoporosis. Safranin-O.[12]

stain with the lectin *Ricinus communis* I (RCA-I).[14] In contrast, other cell types such as mesenchymal and epithelial contain fine fibrillar material or the lysosomes appear empty (Figure 7C), and on paraffin sections they stain with the RCA-I. In addition, there are secondary developmental abnormalities that include dysmyelinogenesis (Figures 12 and 13),[11] which could be due to the interruption of the catabolizing of several glycolipids.[22] The retarded bone formation[12] (Figures 12 and 13), which is similar to Morqueo B disease (MPS-IVB) is due to the interruption of recycling of keratin sulfate.[12] The clinical manifestations include neurovisceral and skeletal alterations with lysosomal storage morphologically. It has been described in DSH, Korat, and Siamese cats, in Alaskan Huskies, Beagles, English Springer Spaniel, Portuguese water dogs, and Shiba dogs, in Friesian cattle, in

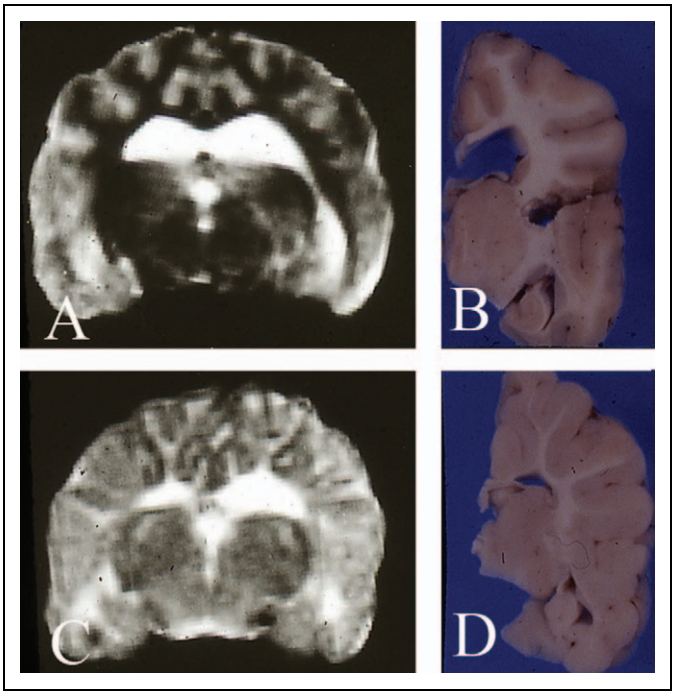

Figure 14. A, T2-weighted magnetic resonance imaging (MRI) of a 9-month-old normal control English Springer Spaniel, illustrating a normal ratio between gray and white matter. B, Corresponding coronal section from the hemisected fixed brain demonstrating prominent gray and white matter. C, MRI of an affected 9-month-old littermate illustrating increased volume of gray matter and poor development of low signal intensity of the white matter. D, Corresponding coronal section revealed increased gray matter and reduced white matter, in particular in the corona radiate.[11]

Coopworth-Romney, Romnet, and Suffolk sheep, and in Emus and American Black bears. It has been suggested that the lysosomal storage of G_{M3} and G_{M1} gangliosides reported in emus is due to the deficiency of sphingolipid activator protein.

G_{M2}-Gangliosidosis

G_{M2}-gangliosidosis is a group of 3 different disorders characterized by primary or secondary deficiency in the activity of lysosomal β-hexosaminidase. There are 2 isoenzymes of β-hexosaminidase, Hex A and Hex B. Three genes are coding proteins that are required for the degradation of G_{M2}-ganglioside. They include the genes that encode α and β subunits of β-hexosaminidase and the G_{M2} activator protein. As a consequence, these 3 disorders differ in their clinical manifestations, in the type of the affected cells, and in the nature of the storage material.[23]

Tay-Sachs Disease

Tay-Sachs disease, often referred to as B variant, is due to a defect in α subunit of Hex A, which results in lysosomal storage of G_{M2}-gangliosides in neural tissue. Clinically, it presents with neurological signs. It has been described in Muntjac deers and in the American flamingos. Ultrastucturally, the neurons contain enlarged lysosomes filled with lamellated membrane structures. Tay-Sachs disease had been described in American flamingo, European Burmese cast, Muntjak deers, Yorkshire pig, Jacob sheep, and raccoons.

Sandhoff Disease

Sandoff disease, often referred to as O variant, is caused by a defect in β subunit that is present in both Hex A and Hex B. This results in lysosomal storage of G_{M2}-gangliosides in neural tissue and oligosaccharides and glycosaminoglycans in mesenchymal and epithelial tissues. Clinically, it is manifested by neurological, visceral, and skeletal changes. Neurons contain numerous lysosomes packed with lamellated membrane structures that often are described as fingerprints or zebra bodies, while other cell types such as mesenchymal and epithelial contain fine fibrillar material or the lysosomes appear empty. It has been reported in DSH, Japanese domestic and Korat cats, German Shorthair, Golden Retriever, Pointer dogs, and in Yorkshire pigs. Four different mutations of the β subunit have been identified in cats.

AB variant

AB variant is due to mutations of the G_{M2} activator protein that is transported to the lysosome via sortilin. The G_{M2} activator protein is required for binding the G_{M2} gangliosides to β-hexoasminidase. Therefore, similar to Tay-Sachs, there is lysosomal storage of G_{M2} gangliosides in neural tissue. Since G_{M2} activator protein is required for binding the G_{M2} gangliosides to β-hexoasminidase, its mutation results in neurological signs. It has been described in Japanese Spaniel dogs and DSH cats.

Galactosialidosis

Galactosialidosis is a lysosomal storage disease that is caused by a defect in the protective protein/cathepsin A that results in the deficiency of β-galactosidase and neuraminidase. The deficiency of both enzymes leads to lysosmal storage of G_{M1}-gangliosides in neural tissue and oligosaccharides in mesenchymal and epithelial tissues. Clinically, the infantile form presents with neurological visceromegaly and skeletal abnormalities.[24] It has been reported in sheep.

Glucocerebrosidosis (Gaucher Disease)

Gaucher disease is primarily characterized by deficient activity of glucocerebrosidase and lysosomal storage of glucocerebrosides[25] and polylactosaminoglycans.[26] Rarely, deficient activity of activator protein (saposin C) causes a similar condition. Saposin C is transmitted to the lysosome via sortilin.[3] Since the major stored substrate originates from the plasma membrane of erythrocytes and leukocytes, reticuloendothelial cells are the most severely affected. The characteristic feature of Gaucher disease is the presence of lipid-laden reticuloendothelial cells (Gaucher cells) in the spleen, liver, and bone marrow. The

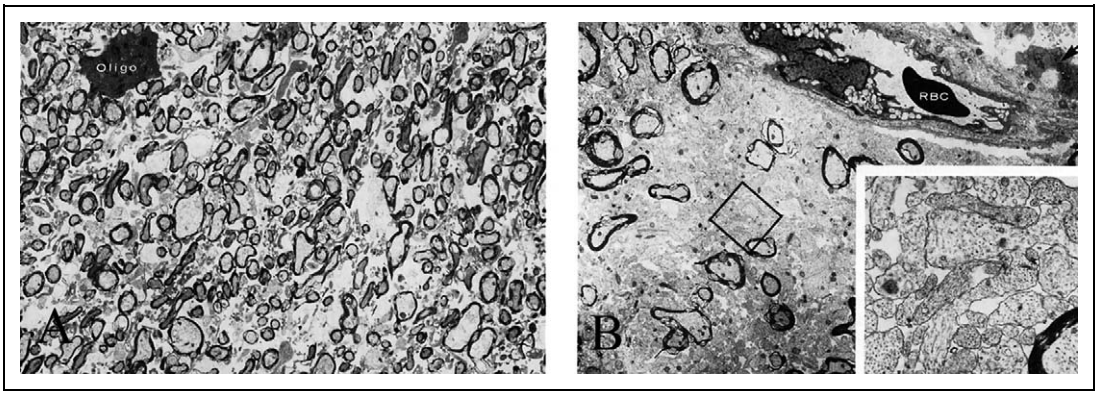

Figure 15. A, Electron micrograph of the corpus callosum from a 2-month-old normal Portuguese water dog (PWD) puppy, illustrating an oligodendrocyte (oligo) and normal myelinated axons. Corresponding electron micrograph from an age-matched affected PWD litter mate demonstrating erythrocyte (RBC), vacuolated endothelial cells, and only few myelinated axons. Inset: Higher magnification of the square revealing normal appearing unmyelinated axons.[11]

Gaucher cells have enlarged striated cytoplasm. Ultrastructurally, Gaucher cells have enlarged lysosomes that contain twisted tubular structures. Gaucher disease has been reported in Silky terrier dogs, pigs, and sheep.

Gaucher-like cells are often observed in disorders in which there is oversupply of substrate, such as myelogeneous leukemia, lymphoma, and various types of hemolytic anemia. In these conditions, there is an oversupply of plasma membrane from white or red cells, which must be catabolized by the reticuloendothelial cells.

Galactosylceramide Lipidosis (Krabbe Disease)

Krabbe disease, also known as globoid cell leukodystrophy, is caused by deficient activity of lysosomal galactosylcerebroside β-galactosidase and lysosomal storage of galactosylsphingosine (psychosine).[27] Rarely, deficient activity of activator protein (saposin A) results in a similar manifestation. Myelinated tissues in the central and peripheral nervous system are most severely affected since galactosylceramide is found almost exclusively in myelin. Morphologically, it is characterized by destruction of myelin and oligodendroglia, glial scarring, and proliferation of globoid cells throughout the white matter. The globoid cells are enlarged vacuolated macrophages typically localized around blood vessels. Ultrastructurally, globoid cells contain enlarged lysosomes packed with twisted tubules (Figure 8). Krabbe disease has been described in Cairn and West Highland white terriers, beagles, blue tick hounds, Pomeranians, Irish setters, miniature poodles, DSH cats, Polled Dorset sheep, ''twitcher'' mice, and rhesus monkeys. In frozen sections, the storage material in the globoid cells is stained with RCA-I lectin that binds to the terminal β-galactosyl residues.

Niemann-Pick Disease Types A and B

Niemann-Pick types A and B are lysosomal storage diseases that result from deficient activity of sphingomyelinase and lysosmal storage of sphingomyelin.[28] Sphingomyelinase is internalized to the lysosome via sortilin.[29] Type A is presented early in life. It is a fatal disorder with severe progressive neurodegeneration and hepatosplenomegaly. In contrast, type B presents later in life and has mild to moderate manifestation. They are presented with hepatosplenomegaly and they have mild or no neurological signs. Morphologically, the affected cells have an enlarged foamy cytoplasm that contains lysosomes packed with lamellated membrane structures. It has been described in poodles and boxer dogs and in Balinese, DSH, and Siamese cats.

Niemann-Pick Type C Disease

Niemann-Pick type C is due to mutations of 2 different genes *NPC1* and *NPC2* that result in a unique error in cellular trafficking of exogenous cholesterol and lead to lysosomal storage of unesterified cholesterol.[30] They code for 2 different proteins NPC1 and NPC2 and comprise different types of cholesterol-binding proteins. The NPC1 is an integral membrane protein localized primarily in late endosomes/lysosomes and also recycled through the Golgi apparatus. The NPC2 is a small soluble protein present in the lysosome; it binds the cholesterol and transfers it to the NPC1 protein that exports the cholesterol from the lysosome to the ER membrane. The clinical manifestations are heterogenous and include severe early onset neurovisceral symptoms as well as mild to moderate late-onset symptoms. Affected cells are enlarged and vacuolated and ultrastructurally contain enlarged secondary lysosomes with heterogenous coarse lamellar structures. It has been described in flight model mice, DSH kittens, and in boxer dogs.

Fabry Disease

Fabry disease is an X-linked recessive disorder caused by deficient activity of lysosomal α-galactosidase in males and often in heterozygous females. This enzyme cleaves the α-galactosyl residues mostly from globotriaosylceramide and to a lesser extent from galactosylceramide.[31] It is thought that the major

mechanism that leads to the clinical manifestations is partly due to poor blood perfusion caused by lysosomal storage in vascular endothelium, particularly in the kidneys, heart, nervous system, and skin. It is noteworthy that other tissues are also involved. The clinical manifestations in heterozygous females are milder. Electron microscopic examination of affected cells reveals enlarged lysosomes containing lamellated membrane structures. On frozen sections, the storage material is stained with *Bandeeirea simplicifolia* I that recognizes terminal α-galactosyl residue.

Farber Lipogranulomatosis

Farber disease is due to the deficient activity of lysosmal acid ceramidase and accumulation of ceramide in lysosomes. It is noteworthy that saposin D is needed to activate the lysosomal ceramidase. Ceramide is an important component in the ganglioside and myelin metabolism. It is characterized by the presence of subcutaneous nodules that are most prominent around joints and also by liver, spleen, and lung granulomas. The granulomas consist of enlarged foamy macrphages and multinucleated giant cells that contain lysosomes. Ultrastructure of the storage material appears as banana-shaped or tubular-shaped vacuoles.[32] Storage material is present in other cell types including neurons and even chondrocytes.

Wolman Disease and Cholesteryl Ester Storage Disease

Both conditions are due to the deficient activity of lysosomal acid lipase and storage of cholesteryl esters and triglycerides in most tissues.[33] The onset of Wolman disease is in infancy. It is severe and is manifested by hepatosplenomegaly, steatorrhea, and adrenal calcification. In contrast, the onset of cholesteryl ester storage disease is later in life; it is milder, and hepatomegaly maybe the only sign. Morphologically, the affected cells contain lysosomes filled with lipid droplets and cholesterol clefts. Wolman disease has been reported in the Sheel parakeet, Donrou rats, and Fox terriers.

Metachromatic Leukodystrophy

Metachromatic leukodystrophy (MLD) is mainly due to the deficient activity of arylsulfatase A and rarely due to the deficiency of activator protein (saposin B).[34] Saposin B is transmitted to the lysosome via sortilin.[3] Arylsulfatase A desulfates the 3-0-sulfogalactosyl from the glycolipids containing 3-0-sulfogalactosyl. These sulfated glycolipids are mostly present in myelin sheaths in the central and peripheral nervous system and less so in the kidney, gallbladder, and liver. Severe neurological defects are manifested in the infantile form; these are moderate to mild in the juvenile and adult forms. Morphologic findings include demyelination in the central and peripheral nervous systems and storage of metachromatic material in neurons and Schwann cells. Metachromatic storage material is present in bile ducts, gallbladder epithelium, hepatocytes, and in segments of the renal tubules. Ultrastructural studies reveal the lysosomal storage of lamellated membrane structures. It had been reported in Hawaian geese.

Multiple Sulfatase Deficiency

Multiple sulfatase deficiency (MSD) is due to mutations of sulfatase-modifying factor 1 (SUMF1).[35] Sulfatase-modifying factor 1 encodes the enzyme responsible for posttranslation modification of a cysteine residue that is essential for the activity of sulfatases. Multiple sulfatase deficiency is characterized by a deficiency of 12 sulfatases and leads to lysosomal storage of sulfated lipids. The clinical manifestations resemble those seen in MLD and MPS-I to MPS-VII. Morphological examinations of neuronal tissue reveals lysosomal storage of lamellated membrane structure, whereas in mesenchymal and some epithelial cells there is storage of a fine fibrillar material.

Glycoprotein Storage Diseases

In this group of storage diseases, oligosaccharides are the major storage compounds, although in some disorders such as fucosidosis there is also storage of glycolipids.[17] Glycoprotein molecules contain oligosaccharide chains covalently attached to a protein. There are 2 major types of glycoproteins, the O-linked glycoproteins that are attached through hydroxyl groups of serine or threonine and the N-linked glycoproteins that are linked through the free amino acid group of asparagines. Glycoproteins are major constituents of cellular membranes and extracellular matrix, including proteoglycan and collagen. Deficient activity of α-fucosidase, α-neuraminidase (sialidase), α-mannosidase, β-mannosidase, β-endo-N-actylglucosaminidase, β-hexosaminidases A and B, which participate in the degradation of oligosaccharides, is manifested in most cell types.

α-Mannosidosis

Deficient activity of lysosomal α-mannosidase results in lysosomal storage and elevated urinary excretion of α-mannosyl-rich *N*-linked oligosaccharides. It has been reported in guinea pigs, Red Angus, Murray Grey, Simmental, Galloway, and shorthorn cattle, DSH, domestic long-haired, and Persian cats. Clinically, they present with severe neurological signs, facial, and skeletal dysmorphia. Morphologically, most cell types have various degrees of foamy, vacuolated cytoplasm and the nature of the storage material is demonstrated by staining with *Concanavalia ensiformis* (Con A; Figure 3).[14] Electron microscopic examination reveals large vacuoles that contain a few fine fibrils. Acquired forms of α-mannosidosis can result from prolonged ingestion of *Swainsona* species or locoweed (*Astragalus lentiginosus*).

β-Mannosidosis

Deficient activity of lysosomal β-mannosidosis results in lysosomal storage and increased urinary secretion of β-mannosyl-rich *N*-linked oligosaccharides. It has been seen in Anglo-

Nubian goats and Salers calves. Clinical manifestations include neurological signs, facial dysmorphism, and joint contracture. Cytoplasmic vacuolization is seen in neural, epithelial, and mesenchymal cells. The vacuoles are stained with PAS and electron-lucent lysosomes are observed under an electron microscopy.

α-Fucosidosis

Fucosidosis is due to the deficient activity of α-fucosidase that cleaves the terminal residues of glycoproteins and oligosaccharides and also cleaves the glycolipids in neural tissues. It had been reported in humans and as an infantile form in English Pointers and as a juvenile form in English Springer Spaniel dogs. Clinically, in both breeds they presented with progressive neuromuscular signs. Morphological studies of dogs revealed vacuolization of neural, epithelial, and mesenchymal cells. The lysosomes in all tissues are electron-lucent/or with very fine fibrils; while in neurons and Schwann cells, the lysosomes contain lamellated membrane structures. Paraffin sections of human tissue are stained with *Ulex europeaus* agglutinin I but not in dog.[36]

Aspartylglucosaminuria

Aspartylglucosaminuria is due to the lack of activity of lysosomal aspartylglucosaminidase (AGU) hydrolase that cleaves the link between aspargine and glycosamine, which is the final step in the breakdown of glycoproteins and oligosaccharides. Deficient activity of AGU results in lysosomal accumulation of aspartylglucosamine. Clinically, it is manifested by developmental delay, skeletal abnormalities, and neurological signs.[37] Morphologically, there is cytoplasmic vacuolation in majority of the cells, and ultrastuctural studies reveal electron-lucent lysosomes or very fine fibrils

Sialidosis

Sialidosis often referred to as mucolipidosis-I is a lysosomal disorder caused by deficient activity of neuraminidase, an enzyme that cleaves α2→3 and α2→6 sialyl linked with several oligosaccharides, glycopeptides, and glycolipids. Clinically, it presents as a severe infantile form with neurovisceral and skeletal abnormalities.[17] Slightly moderate symptoms are manifested later in childhood, while neurological symptoms are characteristic of the adult form. Lysosomes containing lamellated membrane structures are seen in neural tissue while electron-lucent lysosomes or very fine fibrils are seen in mesenchymal and epithelial cells. The storage material stains with wheat germ agglutinin. It has been described in DSH cats.

α-N-acetylgalactosaminidase Deficiency: Schindler Disease

Deficient activity of α-*N*-acetylgalactosaminidase results in lysosomal storage of sialylated and unsialylated glycopeptids, glycosphingolipids, and oligosaccharides with α-*N*-acetylgalactosaminyl residues.[38] Clinically, it is manifested as severe infantile neuroaxonal dystrophy that is moderate to mild in the adult forms. In the infantile form, the morphological changes are limited to the central and peripheral nervous systems. Spheroids are observed under light microscopy. Under electron microscopy, the spheroids are observed to contain tubulovesicular and lamelliform arrays and prominent acicular clefts. In the adult onset form, different cell types, including neural, mesenchymal, and epithelial cells, have vacuolated cytoplasm. Electron microscopic study demonstrated electron-lucent lysosomes or some filamentous material in some type of cells, while others had multilayered electron-dense structures.

Glycogen Storage Disease Type II: Acid α-Glucosidase Deficiency

Glycogen storage disease type II, also referred to as Pompe disease or acid maltase deficiency, is caused by the deficient activity of acid α-glucosidase. This enzyme degrades glycogen and maltase. Deficient activity of acid α-glucosidase results in impaired degradation of glycogen and its lysosomal storage in many tissues, but the largest amounts occurs in cardiac and skeletal muscle and in hepatocytes in severe infantile form. The infantile early-onset form presents with hypotonia, cardiomegaly, rapid progressive weakness, and less marked hepatomegaly. The juvenile and adult onsets are milder and characterized by muscle weakness. Morphologically, the cells are enlarged and vacuolated.[39] On frozen sections, the stored glycogen is stained by PAS and removed by treatment with diastase. Electron microscopy reveals lysosomes packed with fine granular material that is characteristic of glycogen. This disorder had been described in Brahman and Shorthorn cattle and Lapland dogs, cats, Corriedale sheep, and Japanese Quails.

Neuronal Ceroid Lipofuscinosis

The NCL is a heterogenous group of 10 different inherited neurodegenerative lysosomal storage diseases.[40-42] Their clinical manifestations include visual impairment, seizures, progressive psychomotor retardation, and premature death. Histologically, they are characterized by accumulation of autofluorescent material that stains with prostate-specific antigen and Sudan Black in neurons and by a wide variety of other cell types.

Infantile NCL

Infantile NCL (INCL) and late NCL are due to mutations of the *CNL1* gene that codes a soluble lysosomal enzyme, palmitoyl-protein thioesterase 1 (PPT-1). This enzyme removes fatty acids from fatty acylated cysteine residues in proteins. In INCL, the deficient activity of palmitoyl-protein thioesterase results in the storage of saposin A and D. Ultrastructural findings reveal lysosomal storage of GRADs. Deficient activity of PTT-1 has been reported in Swedish sheep and ferrets.

Late-Infantile NCL

Late-infantile NCL is a disorder caused due to mutations of *CLN2*. The gene product is tripeptidyl-peptidase 1 (TPP-1), a lysosomal enzyme. Tripeptidyl-peptidase 1 can release a number of different tripeptides from a free *N*-terminus. In NCL-2, the deficient activity of PPT leads to the storage of subunit c of mitochondrial ATPase synthase. Electron microscopic examinations show storage mixed curvilinear structures. Deficient activity of PTT-1 had been reported in Australian cattle dogs, blue heelers, Border collie, and Dachshund dogs.

Juvenile NCL Batten Disease

Juvenile NCL (JNCL) is due to the mutations of *CNL3* gene that encodes a putative lysosomal membrane protein of unknown function. It is speculated that it plays a role in arginine transport. Mutation of *CNL3* results in the storage of subunit c of mitochondrial ATP synthase. Electron microscopic studies demonstrate lysosomal storage of mixed fingerprint profiles. The NCL-3 had been reported in Chihuahua, Cocker spaniel, Dachshund, Dalmatian, English Setter Saluki, Tibetan terrier, and Yugoslavian shepherd dogs, in Siamese cats, South Hampshire, and Swedish sheep.

Adult NCL (NCL-4)

Adult NCL or Kufs disease is caused by the mutation of gene *CNL4*, and its genotype and the protein it produces are unknown. Mutations of *CNL4* result in the storage of subunit c of mitochondrial ATP synthase. Electron microscopic studies demonstrate lysosomal storage of fingerprints and granular material. The NCL-4 had been reported in American Staffordshire terriers.

Finnish Variant Late Infantile NCL (NCL-5)

The NCL-5 disorder is due to the mutations of the *CNL5* gene that codes soluble lysosomal protein. Mutations of *CNL5* result in the storage of subunit c of mitochondrial ATP synthase. Electron microscopic examinations reveal lysosomal storage of fingerprints, curvilinear, and rectilinear complexes. Mutations of *CNL5* have been characterized in Borderdale sheep and Devon cattle.

Late Infantile Early Juventile NCL (NCL-6)

The NCL-6 disease is caused by mutations of the *CNL6* gene. This gene codes a multi-transmembrane protein that is localized in the ER. The NCL-6 protein interacts with collapsing response mediator protein 2 and plays a role in neuronal maturation. Mutations of NCL-*6* result in the storage of subunit c of mitochondrial ATP synthase. Electron microscopic studies demonstrate lysosomal storage of curvilinear profiles, fingerprints, and rectilinear complexes. The NCL-6 has been reported in South Hampshire, Rambouillet, and Marino sheep, English Setter dogs, Devon cattle, and DSH cats.

Turkish Variant Late-Infantile NCL (NCL-7)

The product of the *CNL7* gene is unknown. Mutations of *CNL7* result in the storage of subunit c of mitochondrial ATP synthase. Electron microscopic studies demonstrate lysosomal storage of mixed fingerprints.

Northern Epilepsy

This condition is due to the mutations of the *CLN8* gene that codes for nonglycosylated transmembrane protein that is present in the ER and the ER-Golgi intermediate compartment. The function of this protein is unknown. Mutations of *CNL7* result in the storage subunit c of mitochondrial ATP synthase and saposin D. Electron microscopic studies demonstrate lysosomal storage of curvilinear profiles or GRAD. Mutation of the *CLN8* gene has been reported in English setter dogs.

Juvenile Variant of NCL (NCL-9)

A new variant of JNCL was recently identified and characterized.[42] It is due to the mutation of *CLN9*, a gene that may be a regulator of dihydroceramide synthase. Electron microscopic examination reveals lysosomal storage of curvilinear profiles, GRADs, and fingerprint profiles.

Cathepsin D deficiency (NCL-10)

Cathepsin D deficiency (NCL-10) is due to the mutation of the cathepsin D gene (*CTSD*). Cathepsin D is a peptidase that belongs to the family of aspartic peptidase and its major function is digestion of proteins and peptides. Deficient activity of CTSD results in lysosomal storage of GRADs mostly in the brain and much less so in visceral organs such as the liver. Lysosomal storage due to mutation of CTSD had been reported in White Swedish Landrace sheep and in American Bulldogs.

Pycnodysostosis: Cathepsin K Deficiency

Cathepsin K is a cysteine protease and is a member of the family of lysosomal proteases that catabolized organic bone matrix and collagen in other tissues. Cathepsin K is internalized into the lysosome via sortilin. Deficient activity of cathepsin K results in defective bone growth and remodeling. It is manifested by short stature, dysmorphism, osteopetrosis, pathologic fractures, and dental abnormalities. Osteopetrosis is the major histological feature.[43] Ultrastructural examinations reveal vacuolated osteoclasts that contain lysosomes with collagen fragments.

Infantile Osteopetrosis and Neuronal Storage Disease

Infantile osteopetrosis and neuronal storage disease is due to mutation of a gene that codes chloride channel 7 (ClC-7). Osteoclasts degrade bone matrix by secretion of acid by vacuolar H^+

adenosine triphosphatase (V-ATPase) and ClC-7.[44] It is followed by the degradation of the bone matrix, mostly collagen type I by cathepsin K. Neuronal storage of PAS and stainable and autofluorescent LFB material is observed. Electron microscopic findings revealed lysosomes containing lamellated membrane structures and electron-dense material and axons packed with spheroids.

Disorders of Membrane Transport

Free sialic acid storage. Lysosomal storage of free sialic acid is due to mutations of a gene that codes for sialin, a lysosomal membrane protein. Sialin is an anion transporter protein that transports free sialic acids to the cytosol. Mutations of sialin result in lysosomal storage of free sialic acids. Clinically, it is manifested as a severe infantile form with severe neurological signs, hepatosplenomegaly, bone dysplasias, and nephrosis. An adult form is milder and is characterized by neurological signs. Morphologically, most cell types have enlarged vacuolated cytoplasm, that is, electron-lucent lysosomes.[45]

Cystinosis. Cystinosis is due to mutations of the gene that codes cystinosin, a lysosomal membrane protein. Cystinosin is a transporter protein that carries the amino acid cysteine from the lysosome to the cytosol. Storage of cysteine occurs in multiple organs including brain, eyes, kidneys, liver, spleen, and thyroids. This results in neurological, visual, thyroid, and renal dysfunction. Light microscopic studies demonstrate cytoplasmic storage of crystals that are birefringent on polarized frozen sections. Electron micrographs demonstrate angular, needle-shaped, and hexagonal crystalline spaces in the cytoplasm.[46]

Danon Disease: Glycogen Storage Disease With Normal Acid Maltase

Danon disease is an X-linked dominant disorder due to mutations of *LAMP-2*, the gene that codes lysosomal membrane protein 2. Originally, it was reported as ''lysosomal glycogen storage disease with normal acid maltase.'' Lysosomal-associated membrane protein 2 is a transmembrane lysosomal protein that participates in chaperone-mediated autophagy and contributes to lysosomal membrane stability and integrity.[47] Clinically, mutations of *LAMP-2* are present as infantile and adult onset forms with mental retardation, myopathy, cardiomyopathy, and retinopathy. Males develop hypertrophic cardiomyopathy, while females present with dilated cardiomyopathy. Affected cells are vacuolated, and electron microscopic examination revealed lysosomes that contain cellular debris and glycogen.

Disorders of Lysosomal-Related Organelles

Genetic, biochemical, and structural studies have revealed that certain specialized cell types have lysosome-related organelles that share features with lysosomes but have distinct morphology, composition, and/or function. They include melanosomes in melanocytes, lytic granules and delta granules in platelets, lamellar bodies in type II pneumocytes, and variants of acidic granules. The most common disorders in the biosynthesis of lysosomal-related organelles is Chediak-Higashi syndrome that have been described in different breeds of cattle, including Brangus, Hereford, and Japanese black, in Persian cats, Killer whales, Aleutian strain of mink and Beige mice. It results in partial albinism, mild bleeding, immune dysfunction, and interstitial pneumonia. Spontaneous mutations in pale ear mice and in cappuccino mice are analogous to the Hermansky-Pudlak syndrome that is associated with severe albinism and very mild bleeding.

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