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Review Article

Lysosomal storage disorders affecting the heart: a review

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ABSTRACT

Lysosomal storage disorders (LSD) comprise a group of diseases caused by a deficiency of lysosomal enzymes, membrane transporters or other proteins involved in lysosomal biology. Lysosomal storage disorders result from an accumulation of specific substrates, due to the inability to break them down. The diseases are classified according to the type of material that is accumulated; for example, lipid storage disorders, mucopolysaccharidoses and glycoproteinoses. Cardiac disease is particularly important in lysosomal glycogen storage diseases (Pompe and Danon disease), mucopolysaccharidoses and in glycosphingolipidoses (Anderson-Fabry disease). Various disease manifestations may be observed including hypertrophic and dilated cardiomyopathy, coronary artery disease and valvular diseases. Endomyocardial biopsies can play an important role in the diagnosis of these diseases. Microscopic features along with ancillary tests like special stains and ultrastructural studies help in the diagnosis of these disorders. Diagnosis is further confirmed based upon enzymatic and molecular genetic analysis. Emerging evidence suggests that Enzyme replacement therapy (ERT) substantially improves many of the features of the disease, including some aspects of cardiac involvement. The identification of these disorders is important due to the availability of ERT, the need for family screening, as well as appropriate patient management and counseling.

Contents

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1. Introduction

Lysosomal storage disorders (LSDs) comprise a group of more than 50 diseases caused by a deficiency of lysosomal enzymes, membrane transporters, or other proteins involved in lysosomal biology. They are caused by lysosomal dysfunction usually as a consequence of deficiency of a single enzyme required for the metabolism of lipids, glycoproteins (sugar-containing proteins), or so-called mucopolysaccharides.

The predominant inheritance pattern is autosomal recessive, except for Anderson–Fabry disease, glycogen storage disease type IIb [Danon disease (DD)], and mucopolysaccharidosis (MPS) type II (Hunter disease). Individually, the LSDs are rare. Overall, their incidence has been estimated as 1 in 7000 to 1 in 8000 live births. Most LSDs are characterized by a progressive course, often resulting in severe disease manifestations and early death [1].

In the late 1950s and early 1960s, de Duve and colleagues identified and characterized the lysosome as a cellular organelle responsible for intracellular digestion and recycling of macromolecules. This was the scientific breakthrough that led to the understanding of the physiological basis of the LSDs [2]. Lysosomes are membrane-bound vesicles that contain digestive enzymes, such as glucosidases, proteases, and sulfatases. The enzymes are synthesized in the endoplasmic reticulum, transported to the Golgi apparatus, and tagged for lysosomes by the addition of mannose-6-phosphate label. Synthesis of lysosomal enzymes is controlled by nuclear genes. Mutations in the genes are responsible for different human genetic disorders, which are collectively known as *lysosomal storage diseases* [1,2].

LSDs result from an accumulation of specific substrates due to the inability to break them down. The diseases are classified according to the type of material that is accumulated, for example, lipid storage disorders, MPS, and glycoproteinoses. This review article focuses on the LSDs that have significant cardiac manifestations. Cardiac disease is particularly important in lysosomal glycogen storage diseases (Pompe and Danon disease), MPS, and glycosphingolipidoses (Anderson–Fabry disease). Various disease manifestations may be observed including hypertrophic and dilated cardiomyopathy, coronary artery disease, and valvular disease (Table 1).

The diagnosis of most LSDs is based mainly on the detection of a specific enzymatic deficiency. In these cases, molecular genetic testing can refine the enzymatic diagnosis. Once the genotype of an individual

Table 1
Lysosomal storage disorders causing cardiac disorders

Diseases	Enzyme deficiency	Cardiac manifestations
Glycogen storage diseases		
Pompe disease	Acid maltase	Massive LVH and cardiac failure
DD	LAMP2	Hypertrophic cardiomyopathy, isolated cardiac variants, progressive conduction system disease
Mucopolysaccharidoses		
Hurler, Schie, Hunter, Sanfilippo, Morquio, Maroteaux–Lamy, Sly, and Natowicz	Alpha-L-iduronidase, iduronate-2-sulfatase, heparan sulfamidase, N-acetylgalactosamine-6-sulfatase, N-acetylgalactosamine-4-sulfatase, beta-D-glucuronidase, hyaluronidase	Valvular regurgitation and stenosis, cardiac hypertrophy, systolic dysfunction
Sphingolipidoses		
Gaucher disease	Beta-glucocerebrosidase	Pulmonary hypertension, cor pulmonale, pericardial effusion, valvular involvement
Niemann–Pick disease	Acid sphingomyelinase	Endocardial fibrosis (rare)
Anderson–Fabry disease	Alpha-galactosidase	Cardiac hypertrophy, progressive conduction dysfunction, arrhythmias, valvular involvement

LSD patient has been ascertained, genetic counseling should include prediction of the possible phenotype and the identification of carriers in the family at risk.

2. Glycogen storage diseases

Glycogen storage diseases affect primarily the liver, skeletal muscle, heart, and sometimes the central nervous system and the kidneys. Glycogen storage diseases are classified according to their individual enzyme deficiency. Each of these enzymes regulates synthesis or degradation of glycogen. Interestingly, there are great phenotypic variation and variable clinical courses even when a specific enzyme is altered by mutation [3]. Lysosomal storage associated with cardiac involvement is found in glycogen storage disease type II a and IIb (Pompe and Danon disease, respectively).

3. Pompe disease (glycogen storage disease type II A)

Pompe disease (glycogen storage disease type II a or acid maltase deficiency) is a lysosomal disorder in which acid α-glucosidase (GAA) deficiencies lead to intralysosomal accumulations of glycogen in all tissues, most notably in skeletal muscles. Pompe disease was first described by Dr. J.C. Pompe in a 7-month-old girl with cardiomyopathy. Massive accumulations of glycogen in vacuoles were observed in all the tissues examined [4]. The connection between lysosomes, the enzyme defect, and Pompe disease was made much later in 1963 by Belgian biochemist Henry Gary-Hers. He discovered a new enzyme that carried out the hydrolysis of glycogen to glucose at an acidic pH and demonstrated that the enzyme was absent in patients with Pompe disease. Dr. Hers realized that this new enzyme resides in the lysosomes and that this is the only glycogen-degrading enzyme present in the lysosomes [5,6].

3.1. Incidence

The incidence of Pompe disease appears to vary by ethnicity and geography. The rapidly progressing infantile-onset form has an estimated frequency of 1:138,000 in white populations, 1:50,000 among Chinese populations, and 1:14,000 among those of African ancestry [7].

3.2. Clinical manifestations

Traditionally, three forms of the disease had been described in the literature based on severity and the time of onset of clinical symptoms. The severity of the disease is related to the degree of enzyme deficiency. Patients have been classified as infantile-, juvenile-, or adult-onset types [8].

3.2.1. Infantile Pompe

Infantile form results from complete or near-complete deficiency of acid GAA. Typically, the disease presents during the first months of life with severe hypotonia, cardiomegaly, macroglossia, and mild hepatomegaly. The age of onset of symptoms is a diagnostic clue, as heart failure usually occurs between 2 and 6 months and always before 18 months. The presentation includes feeding difficulty, cyanosis, dyspnea, sweating, tachycardia, massive cardiac enlargement, susceptibility to respiratory infection, and terminal congestive failure [9]. The patients often present with a respiratory illness prompting a chest radiograph, which usually reveals massive cardiac enlargement. Serum creatine kinase can be elevated but is an unreliable screening tool. Electrocardiogram shows short PR interval, which is pathognomonic of infantile Pompe disease [10]. The echocardiographic findings are distinct because of the increased myocardial mass index (exceeds the value in the most severe forms of familial hypertrophic cardiomyopathy). An unusual echogenicity of the myocardium can be observed and is presumed to relate to the infiltrative process [9].

3.2.2. Juvenile Pompe

The juvenile form is characterized by onset in the first decade. Characteristically, there is reduced but residual GAA activity, and the clinical manifestations are predominantly skeletal muscle weakness with respiratory muscle involvement and mild hepatomegaly. Cardiac involvement is characteristically absent or mild, and death results from respiratory failure after a course lasting several years [8].

3.2.3. Adult Pompe

The adult form of the disease is characterized by onset in the third to sixth decade. It is similar to the juvenile form but is characterized by higher levels of residual GAA activity and a slower progression of the skeletal muscle weakness [8,9].

3.3. Molecular genetics

Pompe disease is an autosomal recessive lysosomal glycogen storage disorder caused by acid GAA deficiency. The *GAA* gene is located on chromosome 17q25. More than 250 mutations have been found on the *GAA* gene. Both copies of the *GAA* gene are needed to harbor a pathogenic sequence variation before the disease manifests itself as partial or complete loss of acid GAA activity. Loss of GAA enzyme activity results in lysosomal glycogen accumulation, which in turn leads to clinical manifestations [11].

3.4. Pathology

In Pompe disease, the heart is grossly enlarged (Fig. 1A). The walls of all chambers are thickened, especially the left ventricular free wall and the papillary muscles (Fig. 1B). In some patients, severe wall thickening is associated with small cardiac cavities and with obstruction to left and/or right ventricular outflow. Other patients show cardiac dilatation. Fibroelastic thickening of the endocardium occurs in about 20% of patients with Pompe disease [9,12].

In histologic sections, there is vacuolar change of cardiac myocyte cytoplasm with a lacework appearance (Fig. 1C). This is caused by massive glycogen deposits which displace the myofibrils to the periphery of the cells. There is paucity of myofibrils and variable degree of interstitial fibrosis [12]. The intracytoplasmic deposits are weakly positive for periodic acid Schiff (PAS) stain (Fig. 1D). These deposits are washed out after diastase treatment. Electron microscopy studies reveal free cytoplasmic accumulation of glycogen in myocytes with granular or fibrillary appearance and loss of myofibrils (Fig. 1E) (Table 2).

Ultrastructural studies reveal large amounts of glycogen accumulate in single-membrane-bound bodies called *glycogenosomes*. In Pompe disease, these have been found in various sites like striated muscle, liver, kidney, skin, pancreas, brain, and eye. However, in striated muscle, much of the glycogen lies outside the lysosomes. It is thought that this might be due to lysosomal rupture by mechanical pressure from muscle contraction. The concomitant release of acid hydrolases would explain the degenerate and necrotic muscle fibers, cell debris, and myelin figures seen with the electron microscope. Occasional single-membrane-bound structures containing glycogen have been seen in muscle tissues on several occasions and not only in Pompe disease [13].

3.5. Treatment

Enzyme replacement therapy (ERT) was approved for human use in 2006. ERT is based on the concept that recombinant lysosomal enzymes can be internalized by cells through the mannose-6-phosphate receptor and then delivered to lysosomes where they are further processed to replace the function of deficient hydrolases. With specific treatment available, there was interest in adding Pompe to the newborn screening program. In March 2015, the US Secretary of Health and Human Services

approved the recommendation to add Pompe disease to the newborn screening program [14].

4. Danon Disease (glycogen storage disease type IIb)

DD is a rare monogenic metabolic X-linked disorder characterized by early-onset cardiomyopathy with hypertrophic or dilated phenotype (frequently responsible for fatal outcome), intellectual disability, and proximal myopathy [15].

4.1. Incidence and prevalence

The exact prevalence of DD is unknown; however, it has been reported in 1%–6% of patients with unexplained left ventricle hypertrophy (LVH) and up to 17% of patients with LVH and other features such as elevated serum creatine kinase or Wolff–Parkinson–White (WPW) syndrome [1,16].

4.2. History

In 1981, Danon and colleagues reported two young boys with a clinical triad of cardiomyopathy, intellectual disability, and myopathy. Skeletal muscle biopsies showed vacuolar alterations reminiscent of type II glycogenosis. No defect in GAA enzyme levels was detected. Since then, similar cases were reported and are usually referred to as *glycogen storage disease without acid maltase deficiency*. It was later recognized that this disease is caused by the primary deficiency of lysosome-associated membrane protein 2 (LAMP2) [17].

4.3. Molecular genetics

DD is caused by the primary deficiency of LAMP2, which coats the inner surface of the lysosomal membrane and is proposed to act as a receptor for proteins to be imported and degraded within lysosomes in chaperone-mediated autophagy. *LAMP2* gene is located on chromosome Xq24 [18]. The disease was considered familial and X-linked because males were predominantly affected. Affected mothers usually had milder and later onset of cardiac symptoms, and male-to-male transmission was not seen [17].

4.4. Clinical manifestations

Clinical manifestations are variable but generally more severe in males because of the X-linked dominance. The cardiac manifestations include LVH, abnormal ECG findings, especially WPW syndrome with very high voltage. These patients can have intellectual disability, skeletal myopathy, and muscle weakness as extracardiac manifestations [19].

4.5. Pathophysiology

Autophagy is a central mechanism in cellular metabolism that cells use to degrade parts of their cytoplasm and organelles using lysosomal enzymes. LAMP2 is required for the conversion of early autophagic vacuoles to vacuoles which rapidly degrade its content. This indicates that LAMP2 may be involved in the process of fusion of autophagic vacuoles with lysosomes which provide the acid hydrolases required for degradation or a function in the maturation of the autophagolysosomes into actively digesting organelles [20].

4.6. Pathology

The heart in DD shows cardiomegaly with biventricular hypertrophy (Fig. 2A). Microscopic examination shows diffuse hypertrophy of the cardiac myocytes with intracytoplasmic vacuoles (Fig. 2B) [21]. The intracytoplasmic deposits are weakly positive for PAS stain. These deposits are washed out after diastase treatment. Immunohistochemistry shows absence of LAMP2 protein expression in cardiac muscle. Electron microscopy shows numerous autophagic vacuoles, correlating with cytoplasmic vacuolization (Fig. 2C) [21].

4.7. Treatment

Catheter ablation is an effective method for the abolishment of WPW syndrome. Implantable cardioverter defibrillator implantation is a preventive treatment for sudden cardiac death. Heart transplantation

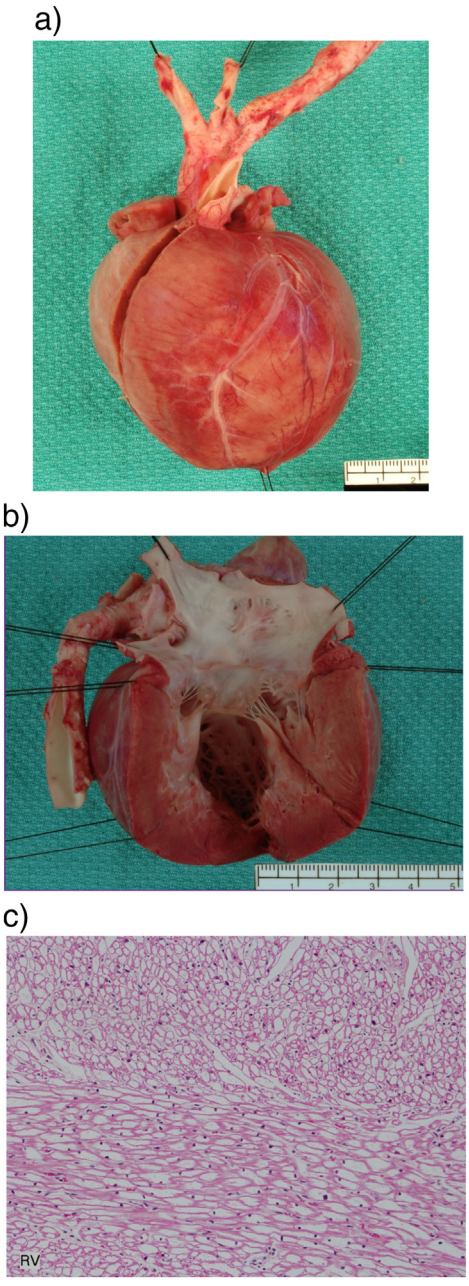

Fig. 1. Pompe disease. (A) Gross image of a heart of a 7-month-old child with Pompe disease shows cardiomegaly with a heart weight of 151 g (expected weight of 42 g). (B) The left ventricle shows concentric hypertrophy with mild endocardial fibrosis (left ventricular wall=16 mm). (C) Microphotograph shows myocardium with marked cytoplasmic vacuolization of myocytes with a lacework appearance [100×, hematoxylin and eosin (H&E)]. (D) Section of the myocardium stained with PAS shows cytoplasmic deposits that are weakly positive with PAS staining. Section stained with PAS diastase (PASD) shows loss of these deposits after diastase treatment (200×, PAS and PASD). (E) Electron micrograph of the myocardium reveals free cytoplasmic accumulation of glycogen in myocytes with granular or fibrillary appearance and loss of myofibrils (5000×).

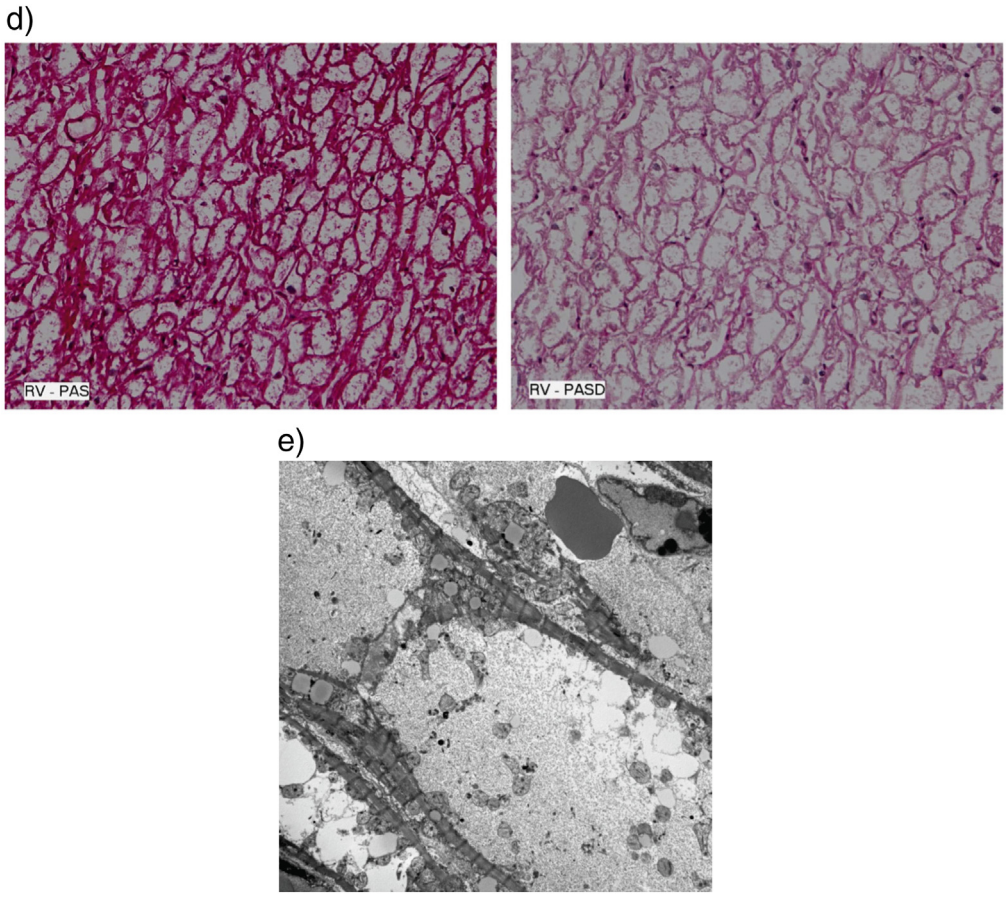

Fig. 1 (*continued*).

significantly enhances the survival of patients with DD. The prognosis is usually poor with early morbidity and limited life expectancy (survival >25 years is uncommon). Death occurs because of heart failure or sudden cardiac death [16].

5. Mucopolysaccharidosis

MPS is a large group of storage diseases caused by a defect of intralysosomal degradation of glycosaminoglycans (GAGs). Seven main forms and several subtypes can be distinguished. Mutations in the *IDUA* gene cause MPS I. Mutations in the *IDUA* gene reduce or completely eliminate the function of the IDUA enzyme. The lack of IDUA enzyme activity leads to the accumulation of GAGs within cells, specifically inside the lysosomes. Deficiency of a-IDUA gives rise to three main classical clinical entities — Hurler syndrome MPS1H, presenting in infancy and the most severe phenotype; Hurler–Scheie syndrome MPS1H/S, a phenotype presenting in childhood of intermediate severity; and Scheie syndrome MPS1S, the mildest form of MPS I. Scheie syndrome MPS1S was formerly named *MPS V*. Cardiomyopathy and thickening of cardiac valves and large vessels have been described in MPS I patients, especially in the most severe form [22].

Table 2
Histochemical, Immunohistochemical and ultrastructural features of Lysosomal storage disorder.

Diseases	Histochemistry & immunohistochemistry	Electron microscopy
Glycogen storage diseases		
Pompe disease	PAS positive PASD negative	Free cytoplasmic accumulation of glycogen in myocytes with granular or fibrillary appearance and myofibrillar loss
DD	PAS positive PASD negative Absence of LAMP2 protein expression in cardiac myocytes	Autophagic vacuoles with glycogen particles
Mucopolysaccharidoses		
Hurler, Schie, Hunter, Sanfilippo, Morquio, Maroteaux–Lamy, Sly, and Natowicz	Alcian blue positive Colloidal iron positive	The storage cells are overloaded with lysosomal residual bodies and membrane-bound inclusion.
Sphingolipidoses		
Gaucher disease	PAS positive	Gaucher cells present (intralysosomal tubular inclusions)
Niemann–Pick disease	Sudan black positive	Intralysosomal myelin-like inclusions
Anderson–Fabry disease	Sudan black B (frozen tissue) positive PAS positive	Aggregates of concentric or parallel lamellae with alternating dense and light bands (myelin figures or zebra bodies)

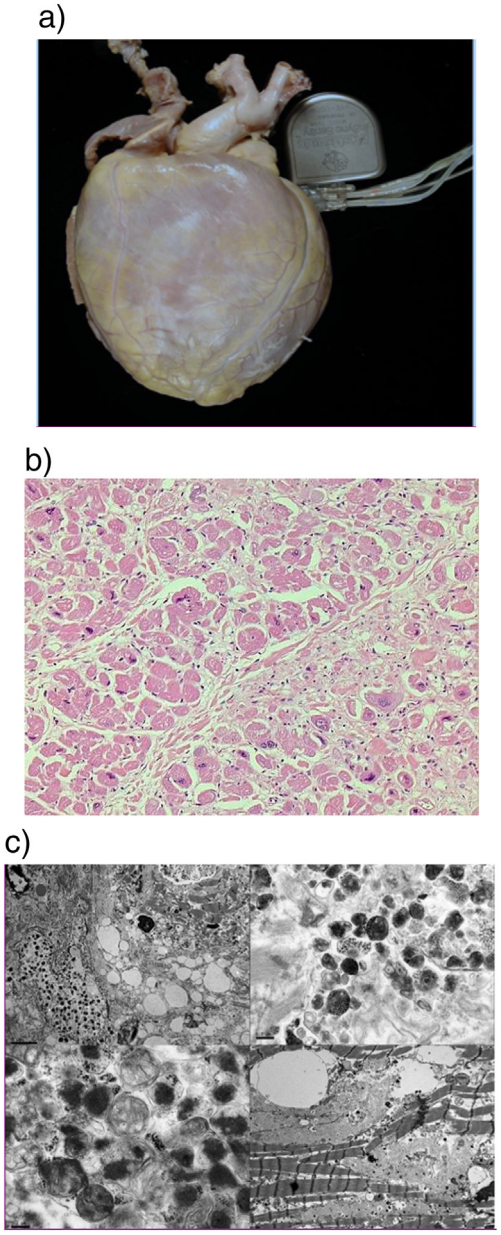

Fig. 2. Danon disease. (A) Gross image of an autopsy heart from a patient with Danon disease shows cardiomegaly (850 g) with biventricular hypertrophy. (B) Microphotograph of the heart shows severe hypertrophy of the myocytes with vacuolar degeneration and interstitial fibrosis (400×, H&E). (C) Electron micrograph of a patient with Danon disease shows numerous autophagic vacuoles interstitial fibroses, increased glycogen deposits, and glycogen deposits in the mitochondria. Scattered lamellar bodies were seen in the cardiac myocyte (15,000×).

Accumulation of GAG results in the clinical features of these disorders, including dysostosis multiplex and coarsening of skin and facial features, as well as central nervous system, respiratory, and cardiac insufficiency [23,24]. Cardiac valve involvement appears more common in those syndromes in which dermatan sulfate catabolism is deranged (MPS I, II, and VI but not MPS III and IV). Most studies have reported that valvular regurgitation is more common. In general, left-sided valves (mitral and aortic) are more severely affected than those on the right side of the heart (tricuspid and pulmonary) [23,24]. The mitral valve leaflets are markedly thickened and cartilage-like, with particularly thickened edges. The subvalvular apparatus of the mitral valve develops shortened chordae tendinea and thick papillary muscles resulting in dysmorphic and poorly mobile leaflets [25]. Calcific deposits in the mitral annular region are also commonly seen. The aortic valve presents a similar morphology and clinical course of progressive valve thickening and dysfunction [26].

Coronary artery narrowing, occlusion, or both have been described in individuals with all types of MPS but are most common in MPS I and MPS II. Diffuse intimal proliferation from GAG deposition within large epicardial coronary arteries can occur early, especially in rapidly progressing MPS I, causing high-grade narrowing [27,28]. In addition, many patients also had abnormally enlarged aortic annuli and sinotubular annuli and sinotubular junction diameters [29].

At radiological examination, there are generalized cardiomegaly and calcification of mitral valve ring. MPS is reported to be the most common cause of mitral annulus calcification in childhood. No specific electrocardiographic pattern is present. Echocardiography may help identify valvular abnormalities and calcific deposits, as well as myocardial abnormalities [8].

5.1. Pathology

Myocardium and blood vessels contain large, oval, or rounded connective tissue cells (Hurler cells) filled with numerous clear vacuoles containing acid mucopolysaccharide material. In addition, small granular cells are present which contain membrane-limited electron dense material associated with fragments of collagen fibrils. The thickening in the tissues is due to the presence of these cells and to an increase in the amount of fibrous connective tissue [12]. These latter cells appear to produce collagen in an abnormal way and are probably responsible for the heavy deposits of collagen in the cardiovascular system of patients with the Hurler syndrome [28].

5.2. Management

Hematopoietic stem cell transplantation (HSCT) has been successfully used for metabolic correction of MPS disorders for the past 30 years [30]. Surgical procedures include mitral valve repair, mitral and aortic replacement, and the Ross procedure. Systemic therapies for MPS include HSCT and ERT [30,31].

6. Sphingolipidoses

Gaucher, Niemann–Pick, and Fabry diseases are the sphingolipidoses that have significant cardiac manifestations.

7. Gaucher disease

Gaucher disease is the most frequent sphingolipidosis. Described originally by Philippe Gaucher in 1882, Gaucher disease is the most prevalent of storage disorders [32]. Mutations in the *GBA* gene cause Gaucher disease. Mutations in the *GBA* gene greatly reduce or eliminate the activity of β-glucocerebrosidase, leading to lysosomal accumulation of glucocerebroside within macrophages. Lipid-laden macrophages (Gaucher cells) accumulate within the reticuloendothelial system, resulting in hepatosplenomegaly, bone marrow replacement, anemia and thrombocytopenia, and skeletal abnormalities [1].

Cardiac manifestations are rare, except in a rare homozygous *D409H* mutation. Marked myocardial infiltration by typical Gaucher cells, causing decreased ventricular compliance and decreased cardiac output, has been reported [12]. Intramyocardial infiltration by Gaucher cells could lead to dilated cardiomyopathy with depressed ejection fraction.

Pulmonary hypertension and cor pulmonale due to occlusion of alveolar capillaries by Gaucher cells derived from the bone marrow have been reported. In few other patients, constrictive calcific pericarditis has resulted from intrapericardial hemorrhage related to the bleeding diathesis that is frequent in Gaucher disease [33].

In 1999, Veinot et al reported the pathological findings of a patient with Gaucher disease, type III C, with prominent cardiac valvular involvement. There were marked fibrosis and calcifications involving the aortic (Fig. 3A) and mitral valve leaflets (Fig. 3B). Microscopic examination showed fibrosis and calcification of the leaflets rimmed by osteoclast-like giant cells. Electron microscopy studies demonstrated the presence of Gaucher cells (Fig. 3C).

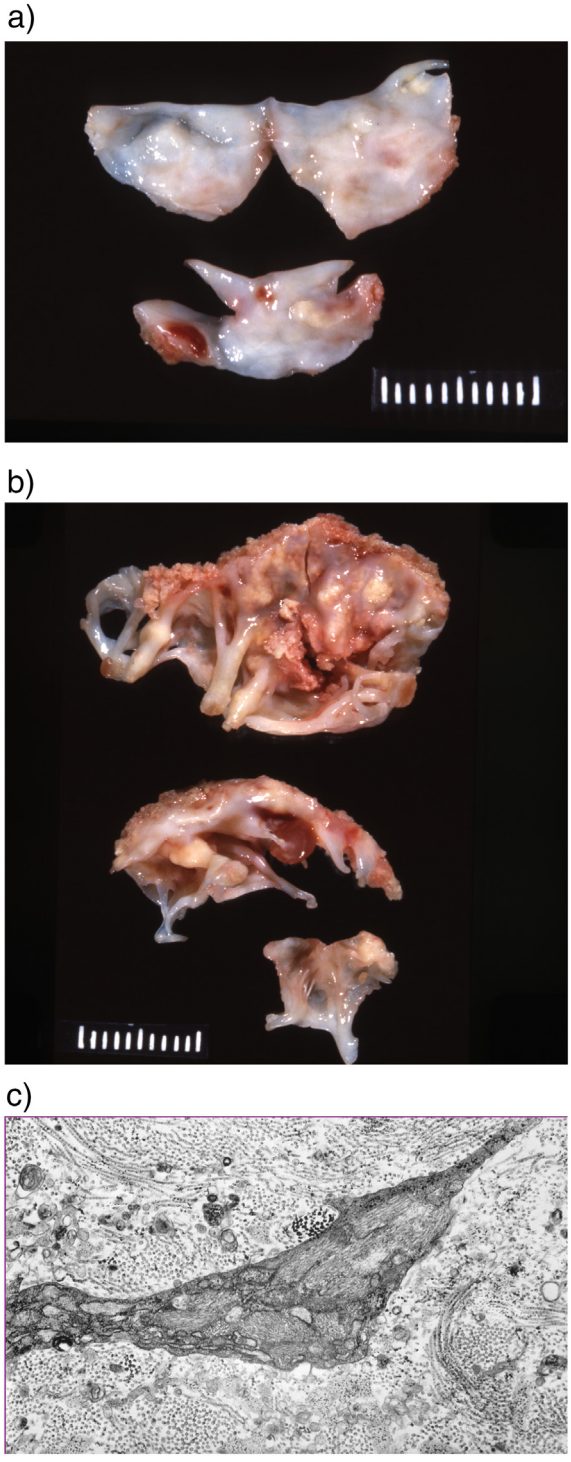

Fig. 3. Gaucher disease. (A) Gross image of an aortic valve from a 17-year-old patient with Gaucher disease shows marked fibrosis and calcification. (B) Gross image of mitral valve leaflet shows marked calcification and surface erosion. (C) Electron microphotograph of a patient with Gaucher disease demonstrates the presence of Gaucher cells (5000×).

8. Niemann–Pick disease

Niemann–Pick disease is caused by a deficiency in acid sphingomyelinase. It is subclassified into type A (infantile, neurodegenerative) and type B (later onset of hepatosplenomegaly, pulmonary involvement, survival into adulthood). Cardiac involvement is rare, usually presenting as endocardial fibrosis [1]. Niemann–Pick disease types A and B are caused by mutations in the *SMPD1* gene. This gene provides instructions for producing an enzyme called *acid sphingomyelinase*.

9. Fabry disease

Fabry disease is an X-linked inheritable deficiency of lysosomal α-galactosidase A (GLA), resulting in an accumulation of neutral glycosphingolipids, mainly globotriaosylceramide (Gb3), in various organ systems. The first descriptions of Fabry disease were made in 1898 by two physicians working independently of each other, William Anderson and Johannes Fabry. The disease is also called *Anderson–Fabry disease* (AFD) after them [34].

9.1. Epidemiology

The incidence of AFD has been estimated at 1 in 40,000 to 1 in 117,000 live births for males. The reported prevalence of AFD in patients with end-stage renal disease on hemodialysis ranges between 0.2% and 1.2% [1].

9.2. Inheritance and molecular genetics

This condition is inherited in an X-linked manner. In males (who have only one X chromosome), one altered copy of the *GLA* gene in each cell is sufficient to cause the condition. The *GLA* gene consists of seven exons located on the long arm of the X chromosome (Xq22.1). More than 250 mutations have been described in all seven exons, the majority of which are missense point mutations [1,35].

9.3. Clinical manifestations

Angiokeratosis and corneal opacities are common. Cardiovascular manifestations include LVH and cardiac failure. Systemic arterial hypertension is common, generally due to renal failure. Angina pectoris and myocardial infarction may occur [9]. Gb3 accumulates in all cardiac cell types, including microvascular endothelial and smooth muscle cells, fibroblasts, and cardiomyocytes, leading to myocardial ischemia, valve abnormalities, and myocardial hypertrophy that mimic the morphological and clinical picture of hypertrophic cardiomyopathy or unexplained LVH [36]. The 2 main types of disease manifestations include the classic variant and the cardiac variant.

In the classic variant, males have very low or absent α-Gal A activity, which results in severe systemic manifestations that typically begin in childhood or adolescence and include acroparesthesias, angiokeratomas, cornea verticillata, hyperhidrosis, mild proteinuria, and gastrointestinal problems [37,38]. Cardiac involvement typically becomes noticeable between 20 and 40 years of age, and renal involvement usually progresses to the point of requiring dialysis or renal transplantation [39]. Heart failure, arrhythmias, and myocardial infarction are usually late manifestations [40]. Cerebrovascular manifestations include early stroke, thromboses, and transient ischemic attacks, which lead to significant neurological deterioration and death [39,40].

Patients with the “cardiac variant” of Fabry disease have residual enzyme activity (approximately 1%–5% of normal values). They present in the fifth and sixth decades of life with unexplained LVH and conduction disease, without other classic manifestations of Fabry disease. Histologically, patients with the cardiac variant are different from those

with classic Fabry disease in that there is an absence of vascular endothelial glycosphingolipid deposits [41].

Cardiac involvement is frequent in Fabry disease. Patients develop LVH, arrhythmias, conduction abnormalities, and valvular abnormalities and may develop coronary heart disease [42]. Gb3-mediated infiltration and microvascular damage in AFD lead to restrictive pathophysiology and concentric hypertrophy of the heart. This has emerged as the leading cause of mortality in patients with AFD. Diastolic dysfunction and progressive LVH comprise the major features of cardiac involvement in AFD [43].

9.4. Pathology

The heart shows cardiomegaly with asymmetric septal hypertrophy or concentric hypertrophy. The papillary muscles are prominent. There are associated mitral and aortic valve abnormalities including mitral stenosis, aortic regurgitation, and mitral regurgitation.

In histologic sections of the myocardium, the deposits of ceramide trihexoside appear as vacuoles (Fig. 4A and B). In frozen sections, they are sudanophilic, PAS positive, and strongly birefringent (Fig. 4C). In cardiac muscle cells, these deposits occupy the central, perinuclear areas, displacing the contractile elements toward the periphery. This results in a lacework histologic appearance.

Ultrastructural study reveals that the ceramide trihexoside deposits form intralysosomal aggregates of concentric or parallel lamellae spaced 4 to 5.5 nm apart (Fig. 4D). Their birefringence in frozen sections and their highly organized substructure serve to differentiate the lamellar deposits in Fabry disease from the irregular arrays of concentric lamellae that often are encountered as nonspecific findings in degenerated cardiac muscle cells [12].

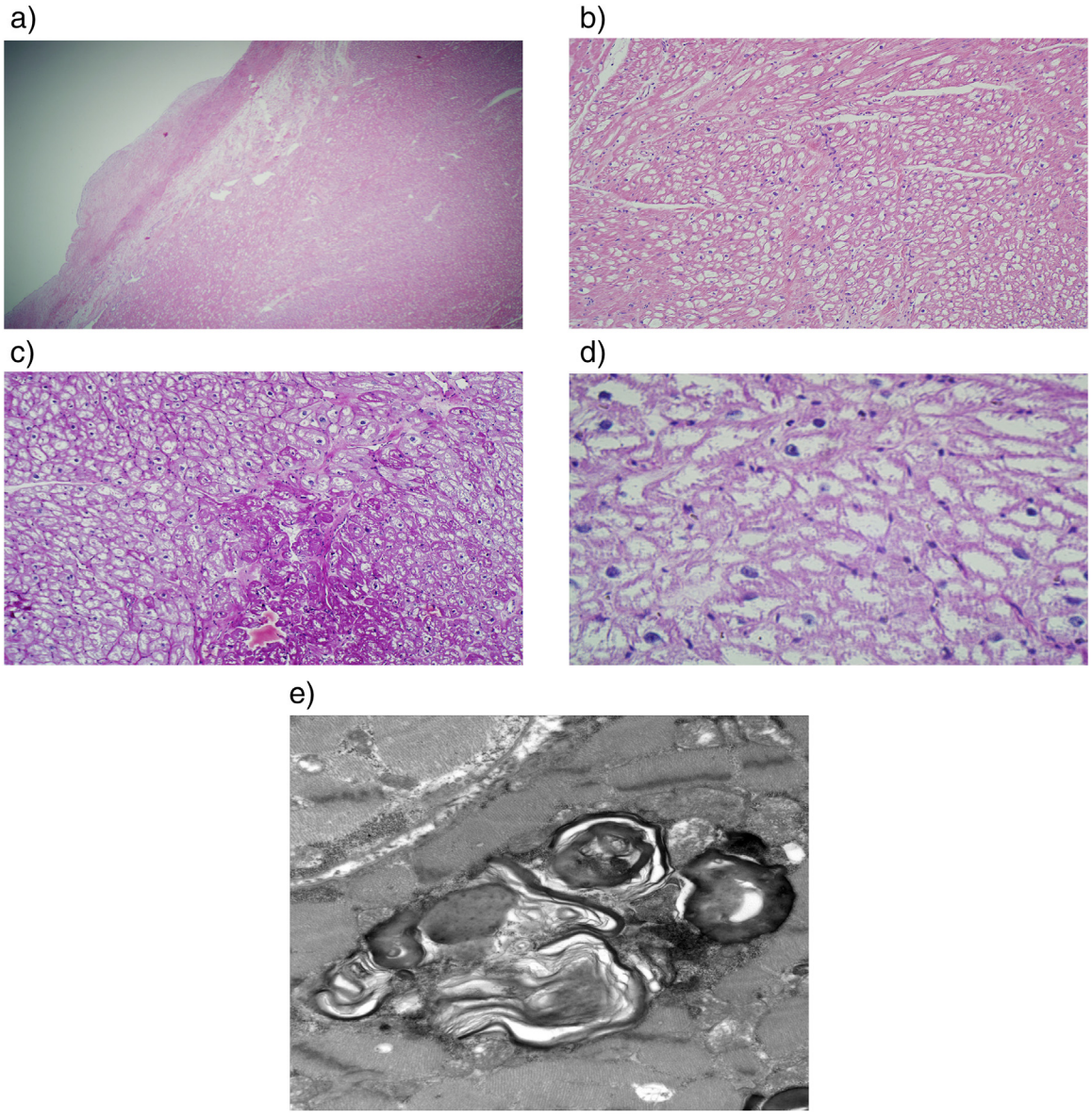

Fig. 4. Fabry disease. (A) Microphotograph of a septal myectomy specimen from a patient with Fabry disease shows endocardial fibrosis (160×, H&E). (B) A higher magnification shows cardiac myocytes with diffuse cytoplasmic vacuolization (200×, H&E). (C) Microphotograph showing PAS-positive deposits. In cardiac muscle cells, these deposits occupy the central, perinuclear areas, displacing the contractile elements toward the periphery (200×, PAS). (D) Section stained with PASD shows loss of these deposits after diastase treatment (200×, PASD). (E) Electron microphotograph of a patient with Fabry disease shows ceramide trihexoside deposits form intralysosomal aggregates of concentric or parallel lamellae spaced 4 to 5.5 nm apart (20,000×).

9.5. Treatment modalities

General measures include β-blockers, calcium channel blockers, and antiplatelet conventional treatment with angiotensin-converting enzyme inhibitors and diuretics.

In 2001, two ERTs were released: agalsidase alpha and agalsidase beta. ERT is not a cure but can allow normal metabolism, and both prevent disease progression as well as potentially reverse symptoms. The high cost of the drugs presents a barrier to usage in many patients [44,45]. Early intervention with ERT slows progression of disease. Cardiomyopathy is a common cause of morbidity. Death occurs at a mean age of 50 years in men and 70 years in women.

9.6. Differential diagnosis of lysosomal storage diseases affecting the heart

9.6.1. Hydroxychloroquine toxicity

Hydroxychloroquine and its predecessor chloroquine are medications commonly used in the treatment of systemic lupus erythematosus, rheumatoid arthritis, and other connective tissue disorders. Hydroxychloroquine interferes with malarial metabolites, confers immunomodulatory effects, and also affects lysosomal function [46].

Clinical manifestations of antimalarial-induced cardiotoxicity can manifest as restrictive cardiomyopathy, dilated cardiomyopathy, or conduction abnormalities such as bundle branch and atrioventricular block. Patients usually present with heart failure symptoms [47]. On echocardiography, the hallmark finding of hydroxychloroquine cardiotoxicity is a diffusely thickened ventricular wall. Microscopic examination of the endomyocardial biopsy shows abnormal vacuoles in the cardiomyocytes (Fig. 5A). The vacuoles are PAS negative. Electron microscopy demonstrates myelinoid bodies, vacuolated cytoplasm with cell debris (Fig. 5B), and curvilinear bodies (Fig. 5C) [46,47] (Table 3).

9.6.2. PRKAG2 mutation-related cardiomyopathy

This is a rare autosomal dominant glycogen storage disorder and is characterized by cardiac hypertrophy, arrhythmias, and conduction defects. Glycogen accumulation is due to cellular uptake of glycose as opposed to a defect in glycogen degradation. Mutations in the gene for gamma 2 regulatory subunit of AMP-activated protein kinase (*PRKAG2*) cause glycogen accumulation [48].

PRKAG2 syndrome leads to pronounced enlargement of myocytes with frequent intracellular vacuoles filled with glycogen (Fig. 6A). Toluidine blue can show the intracellular glycogen accumulation in both the vacuoles and subsarcolemma. Electron microscopy indicates intracellular glycogen accumulation and occasional myelin bodies (Fig. 6B) [49].

The presence of hypertrophic cardiomyopathy in the setting of WPW syndrome raises the suspicion for an underlying genetic disorder. Genetic testing for mutation in *PRKAG2* gene can confirm the clinical diagnosis of this glycogen storage disorder [48,49].

9.6.3. Mitochondrial cardiomyopathy

Mitochondrial cardiomyopathy is described as a myocardial condition characterized by abnormal heart muscle structure, function, or both, secondary to genetic defects involving the mitochondrial respiratory chain. The myocardial dysfunction is seen in the absence of concomitant coronary artery disease, systemic arterial hypertension, valvular disease, or congenital heart disease. The typical cardiac manifestations of mitochondrial disease include hypertrophic and dilated cardiomyopathy, arrhythmias, left ventricular myocardial noncompaction, and heart failure [50].

Microscopic features that suggest cardiomyopathy in mitochondrial disease include fusiform enlargement of affected myocytes around the perinuclear region with cytoplasmic clearing and replacement of cross striae by fine granules. Ultrastructural examination shows abnormal mitochondria with ring mitochondria, giant mitochondria with membrane fusion and circular cristae, concentric cristae, and giant organelles with irregularly whorled and undulated cristae [51]. Genetic studies show both deletions and tRNA point mutations involving mitochondrial (mt DNA) [50].

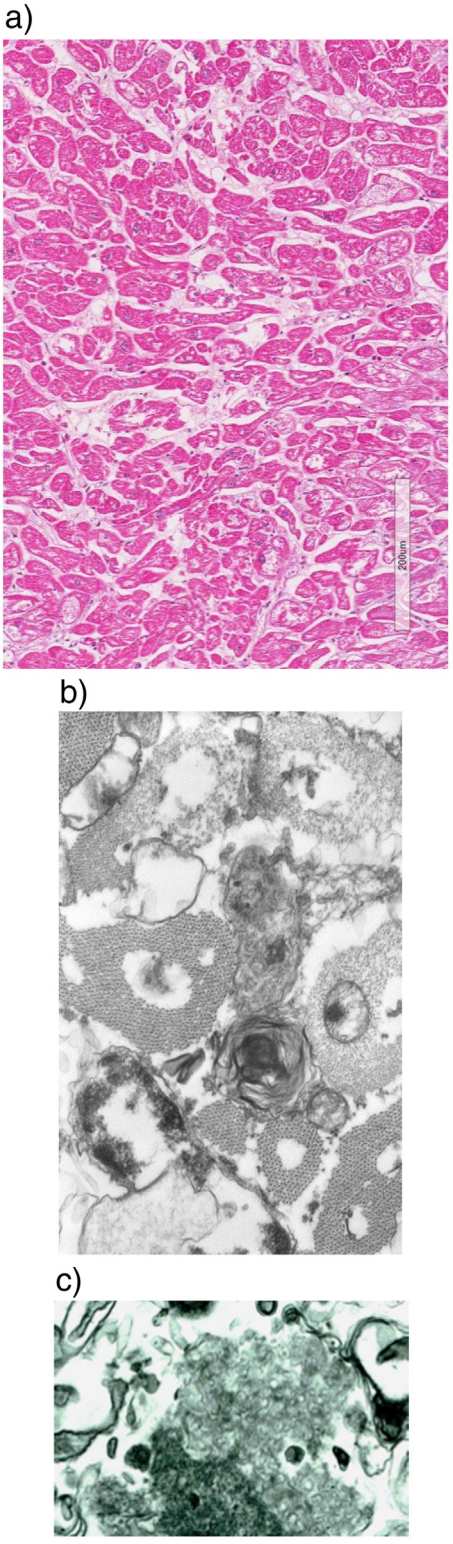

Fig. 5. Hydroxychloroquine toxicity. (A) Light microscopy image of an endomyocardial biopsy demonstrates abnormal vacuoles in cardiomyocytes (200×, H&E). (B) Electron microphotograph of cardiac myocytes demonstrates myeloid bodies and vacuolated cytoplasm with cell debris (12,000×). (C) Electron microphotograph of cardiac myocytes demonstrates curvilinear bodies (20,000×).

Table 3
Differential diagnosis of Fabry Disease.

Disease	Pathogenesis	Pathology	Electron microscopy
Fabry disease	Deficiency of lysosomal alpha-galactosidase A	Myocyte enlargement with vacuoles with sudanophilic, PAS-positive material	Aggregates of concentric and parallel lamellae with alternating dense and light bands
Hydroxychloroquine toxicity	Interference with lysosomal function	Myocyte enlargement with vacuoles with PAS-negative material	Myelinoid bodies, curvilinear bodies, and vacuolated cytoplasm with cell debris
PRKAG2-related cardiomyopathy	AMP-activated kinase PRKAG2 mutation	Myocyte enlargement with intracellular glycogen accumulation	Intracellular glycogen and myelin bodies
Mitochondrial cardiomyopathy	Genetic defects in mitochondrial respiratory chain	Fusiform enlarged myocyte with perinuclear cytoplasmic clearing	Abnormal ring and giant mitochondria with membrane fusion and circular cristae
Hypertrophic cardiomyopathy	Mutations in sarcomeric genes	Myocyte hypertrophy and disarray	Myofibrillar disarray

9.6.4. Hypertrophic cardiomyopathy

Hypertrophic cardiomyopathy (HCM) is defined by the presence of increased left ventricular wall thickness in a nondilated chamber that is not solely explained by abnormal loading conditions [52]. Clinical characteristics include LVH; diastolic dysfunction; and increased risk for arrhythmias, sudden death, and heart failure [53].

The heart shows ventricular hypertrophy that is characteristically asymmetric with disproportionate septal thickening, particularly at the confluence of the anterior septum and anterior free wall. Symmetric, apical, and other atypical distributions of hypertrophy are also described. Mitral leaflet abnormalities include enlargement or elongation and presence of accessory conduction tissues.

Microscopic examination shows that the cardiomyocytes are hypertrophied with abundant eosinophilic cytoplasm and box-shaped nuclei (Fig. 7A). Myocardial architecture is disorganized, with bundles of cardiomyocytes arranged at perpendicular and oblique angles to each other (myocardial disarray) (Fig. 7B). Replacement myocardial fibrosis resulting from microvascular ischemia and resultant cell death may be seen. Ultrastructural examination shows characteristic microfibrillar disarray [12,54].

Pathogenic variants for HCM have been described in eight genes encoding sarcomere proteins, with most present in the *MYH7* and *MYBPC3* genes. Collectively, sarcomere variants are identified in up to 60% of patients with HCM who also have a family history and in up to 40% of patients with sporadic HCM. Storage cardiomyopathies masquerading as HCM are caused by mutations in *LAMP2* (DD), *PRKAG2* (WPW syndrome), and *GLA* (Fabry disease). Recent clinical guidelines for HCM recommend comprehensive testing for five HCM genes (*MYBPC3, MYH7, TNNI3, TNNT2*, and *TPM1*). Sequencing panels including, but not limited to, these genes are offered by several laboratories in the United States and worldwide [55].

The initial therapy for symptomatic patients with obstruction is medical therapy with β-blockers and calcium antagonists. However, there remain subsets of patients who have continued severe symptoms, which are unresponsive to medical therapy. These patients can be treated with septal reduction therapy, either surgical septal myectomy or alcohol septal ablation [56].

9.7. Biochemical and molecular genetic testing

Biochemical and genetic diagnosis of LSDs should be performed in specialized laboratories. Various clinical samples can be used for analysis, such as blood, urine, amniotic fluid, skin fibroblasts, and tissue biopsies. The tests performed for diagnosis can be divided into categories outlined below [57].

9.7.1. Urinary oligosaccharides

Urinary oligosaccharide screening is performed by high-performance thin-layer chromatography using the technique. This method, however, has severe limitations. Definitive identification of specific metabolites is not possible [57,58].

9.7.2. Urinary glycosaminoglycans

Mucopolysaccharidoses may be suspected from measurements of urinary GAGs. This method assesses the amount of hexuronic acid contained in the extracted GAGs and can give false-negative results when the accumulated GAG is keratin sulfate, which contains galactose instead of hexuronic acid; in this case, the diagnosis of Morquio disease may be missed [57].

9.7.3. Assessment of specific substrates

The development of tandem mass spectrometry for the identification and quantification of lysosomal substrates and metabolites has been a significant advance in the diagnosis of LSDs [59,60]. In almost all cases, glycosphingolipids and oligosaccharides analyzed by this method have been shown to differ significantly in controls and affected patients [57,59]. The development of tandem mass spectrometry led to a major shift in the approach to newborn screening of LSDs [14].

9.7.4. Assessment of lysosomal enzyme activities

Lysosomal enzyme activities are usually determined by a fluorometric assay in cultured fibroblasts, leukocytes, or sera. The activity of another lysosomal enzyme should also be assayed as a control for cell integrity. LSDs can be associated with either low or undetectable enzyme activity. In some diseases, a correlation has been found between the level of residual enzyme activity and phenotypic severity [57,61].

In Pompe disease, GAA enzyme activities of less than 1% of normal controls are seen in the infantile form. Both the American College of Medical Genetics and the Japanese guidelines for the management of Pompe disease recommend a GAA assay performed on skin fibroblasts (preferred tissue) or skeletal muscle biopsy as the diagnostic "gold standard" [62–64].

α-Gal A activity assays of plasma or leukocytes can be used to diagnose Fabry disease. Activity <5% of mean in men is highly suggestive of classic AFD. Many patients, however, might have markedly reduced α-Gal A activity of uncertain clinical significance. In women in particular, plasma α-Gal A activity is highly variable and can be normal even in the presence of clinical disease. Therefore, confirmatory genetic testing is necessary in many cases involving either sex [44,45].

9.7.5. Indirect biomarkers

Indirect biomarkers may be useful for the identification of LSDs and for monitoring the effects of treatment. For example, increased plasma levels of two molecules, chitotriosidase and CCL18/pulmonary and activation regulated chemokine, have been reported in patients with Gaucher disease [57].

9.7.6. Molecular genetics

Molecular genetic testing can clarify the type of genetic variation and its impact on the protein and on the presence of residual enzyme activity. This information is crucial in evaluating treatment options, such as ERT, to date only available for some disorders, and alternative

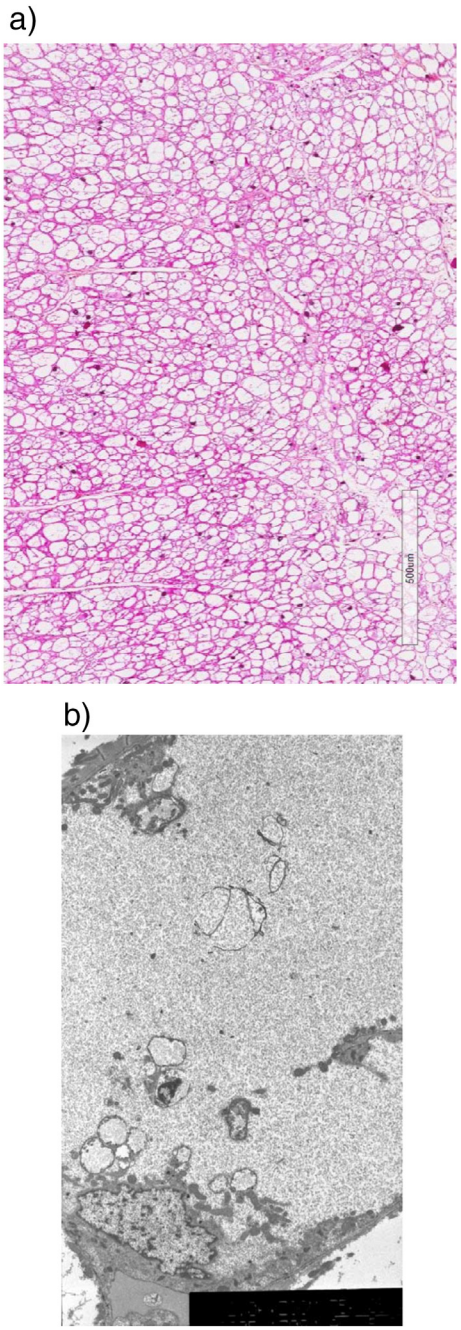

Fig. 6. PRKAG2 syndrome. (A) Microphotograph of myocardium from a patient with PRKAG2 syndrome shows cardiac myocytes with diffuse cytoplasmic vacuolization (50×, H&E). (B) Electron microphotograph of cardiac myocytes shows marked cytoplasmic glycogen accumulation within the cell.

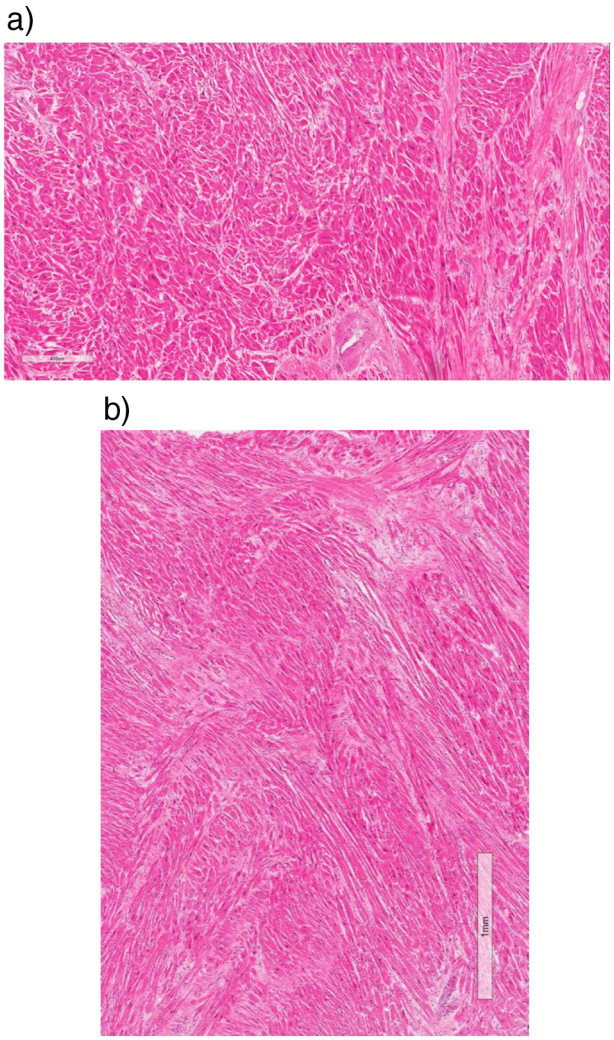

Fig. 7. Hypertrophic cardiomyopathy. (A) Microphotograph of myocardium shows diffusely hypertrophied cardiomyocytes with abundant eosinophilic cytoplasm and enlarged nuclei (100×, H&E). (B) Microphotograph shows myocardium with bundles of cardiomyocytes arranged at perpendicular and oblique angles to each other (myocardial disarray) (50×, H&E).

treatments such as pharmacological chaperones or substrate reduction therapy (SRT), for which clinical trials are still in progress. Identification of the mutations responsible for the LSDs has facilitated understanding of the pathophysiology of these diseases. It also enables prenatal and postnatal testing and allows the provision of genetic counseling [65]. In general; the interpretation of a molecular result should depend on a comprehensive evaluation that includes related clinical, paraclinical, and biochemical data.

LSDs are monogenic diseases, the majority of which have an autosomal recessive mode of inheritance. To date, X-linked inheritance has been observed in Hunter disease, Fabry disease, and DD. Genetic testing is an important component in the diagnosis of AFD. When a mutation in the GLA gene of a patient is identified, it should be compared with known mutations in the literature to verify whether it is associated with the classic phenotype or variant phenotype, or if it is a genetic variant of unknown significance [43].

Molecular sequencing of the acid α-glucosidase (*GAA*) gene is important for confirmatory (second-tier) testing after a positive newborn screen for Pompe disease. Because screening laboratories generally do not have sequencing capabilities, second-tier screening is not part of most New Born Screening (NBS) programs. A number of NBS programs, however, do have second-tier screening that includes full gene sequencing [14].

Targeted Next-Generation Sequencing (NSG)-based gene panels for inherited cardiomyopathies are offered by a growing number of laboratories worldwide. These tests provide a comprehensive analysis of the genes associated with inherited cardiomyopathy conditions like hypertrophic, dilated, restrictive, and left ventricular noncompaction cardiomyopathy. Some of the panels include cardiomyopathies associated with other diseases including LSDs like Fabry disease, DD, and glycogen storage diseases.

9.7.7. Treatment modalities for LSDs

The first clinical use of ERT was developed for Gaucher disease patients and received FDA approval in 1991. ERT was later developed for Pompe disease and then Fabry disease [45].

SRTs attempt to treat LSDs by inhibiting the production of the substrate that accumulates in the respective LSD. The first SRT that was developed decreased the amount of glucocerebroside produced, thereby indirectly lessening the accumulation of the unwanted substrate. Small-molecule drugs commonly called *substrate reduction therapies* inhibit the production of the substrate that accumulates in the respective LSD [31,66].

HSCT may be effective under optimal conditions in preventing the progression of central nervous system symptoms in neuronopathic forms of lysosomal storage diseases, including some of the mucopolysaccharidoses, oligosaccharidoses, sphingolipidoses, and lipidoses [66,67].

Gene therapy may have the highest potential to treat or cure LSDs because it has the potential to directly address the genetic makeup of these diseases. Most gene therapy approaches attempt to "add back" genetic information missing in LSD patients. Currently, there are three classes of viral vectors used in clinical and preclinical trials attempting to deliver putative LSD corrective genes: adenovirus, adeno-associated virus, and retrovirus/lentivirus. Despite the high potential, development of gene therapies for LSDs has been slow moving because of the many novel obstacles [57].

10. Conclusion

LSDs are a group of distinct genetic disorders, each of which is the result of a specific defect in a lysosomal enzyme. They should be considered in the differential diagnosis of children and adults presenting with cardiac hypertrophy, coronary artery disease, or valvular disease. Endomyocardial biopsies can play an important role in the diagnosis of these diseases. Microscopic features along with ancillary tests like special stains and ultrastructural studies help in the diagnosis of these disorders. Diagnosis is further confirmed based upon enzymatic and molecular genetic analysis. Emerging evidence suggests that ERT substantially improves many of the features of the disease, including some aspects of cardiac involvement. The identification of these disorders is important due to the availability of ERT, the need for family screening, as well as appropriate patient management and counseling.

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References

[1] Linhart A, Elliott PM. The heart in Anderson–Fabry disease and other lysosomal storage disorders. Heart 2007;93(4):528–35 [Review. PubMed PMID: 17401074; PubMed Central PMCID: PMC1861503].
[2] de Duve C. The lysosome turns fifty. Nat Cell Biol 2005;7(9):847–9. https://doi.org/10.1038/ncb0905-847 [PMID 16136179].
[3] Hicks J, Wartchow E, Mierau G. Glycogen storage diseases: a brief review and update on clinical features, genetic abnormalities, pathologic features, and treatment. Ultrastruct Pathol 2011;35(5):183–96. https://doi.org/10.3109/01913123.2011.601404 [Review. PubMed PMID: 21910565].
[4] Kroos M, Pomponio RJ, van Vliet L, Palmer RE, Phipps M, Van der Helm R, et al. Reuser A; GAA Database Consortium. Update of the Pompe disease mutation database with 107 sequence variants and a format for severity rating. Hum Mutat 2008;29(6):E13–26. https://doi.org/10.1002/humu.20745 [PubMed PMID: 18425781].
[5] Lim JA, Li L, Raben N. Pompe disease: from pathophysiology to therapy and back again. Front Aging Neurosci 2014;6:177. https://doi.org/10.3389/fnagi.2014.00177 [eCollection 2014. Review. PubMed PMID: 25183957; PubMed Central PMCID: PMC4135233].
[6] Dasouki M, Jawdat O, Almadhoun O, Pasnoor M, McVey AL, Abuzinadah A, et al. Pompe disease: literature review and case series. Neurol Clin 2014;32(3):751–776, ix. https://doi.org/10.1016/j.ncl.2014.04.010 [Review. PubMed PMID: 25037089; PubMed Central PMCID: PMC4311397].
[7] Chien YH, Hwu WL, Lee NC. Pompe disease: early diagnosis and early treatment make a difference. Pediatr Neonatol 2013;54(4):219–27. https://doi.org/10.1016/j.pedneo.2013.03.009 [Epub 2013 Apr 28. Review. PubMed PMID: 23632029].
[8] Raben N, Plotz P, Byrne BJ. Acid alpha-glucosidase deficiency (glycogenosis type II, Pompe disease). Curr Mol Med 2002;2(2):145–66 [Review. PubMed PMID: 11949932].
[9] Wenger NK, Goodwin JF, Roberts WC. Cardiomyopathy and myocardial involvement in systemic disease. In: Hurst WH, editor in chief, Logue RB, Rackely CE, Schlant RC, Sonenblick EH, Wallace AG, Wenger NK, editors. Hurst's the heart, 6th ed: McGraw Hill Book Company; 1986, p.1181–1249.
[10] Mazzitello WF, Briggs JF. Glycogen storage disease of the myocardium. Dis Chest 1957;32(6):636–45 [PubMed PMID: 13480207].
[11] Kroos M, Hoogeveen-Westerveld M, van der Ploeg A, Reuser AJ. The genotype-phenotype correlation in Pompe disease. Am J Med Genet C Semin Med Genet 2012;160C(1):59–68. https://doi.org/10.1002/ajmg.c.31318 [Epub 2012 Jan 17. Review. PubMed PMID: 22253258].
[12] Lester Wanda M, Avrum I. Cardiovascular effects of systemic diseases and conditions. In: Silver Malcolm, Gotlieb Avrum I, Schoen Frederick R, editors. Cardiovascular pathology. 3rd ed. Pennyslvannia: Churchill Livingston; 2001. p. 493–540.
[13] Ghadially FN. Lysosomes. In: Ghadially Feroze N, editor. Ultrastructural pathology of the cell and matrix, volume 2. 4th ed. Masschussets: Butterworth- Heineman; 1997.
[14] Bodamer OA, Scott CR, Giugliani R, Pompe Disease Newborn Screening Working Group. Newborn screening for Pompe disease. Pediatrics 2017;140(Suppl.1):S4–S13. https://doi.org/10.1542/peds.2016-0280C [PubMed PMID: 29162673].
[15] Bottillo I, Giordano C, Cerbelli B, D'Angelantonio D, Lipari M, Polidori T, et al. A novel LAMP2 mutation associated with severe cardiac hypertrophy and microvascular remodeling in a female with Danon disease: a case report and literature review. Cardiovasc Pathol 2016;25(5):423–31. https://doi.org/10.1016/j.carpath.2016.07.005 [Epub 2016 Jul 25. Review. PubMed PMID: 27497751].
[16] D'souza RS, Mestroni L, Taylor MRG. Danon disease for the cardiologist: case report and review of the literature. J Community Hosp Intern Med Perspect 2017;7(2):107–14. https://doi.org/10.1080/2000966620171324239 [eCollection 2017 Mar. PubMed PMID: 28638575; PubMed Central PMCID: PMC5473185].
[17] Danon MJ, Oh SJ, DiMauro S, et al. Lysosomal glycogen storage disease with normal acid maltase. Neurology 1981;31:51–7.
[18] Di Mauro S, Tanji K, Hirano M. LAMP-2 deficiency (Danon disease). Acta Myol 2007;26(1):79–82 [PubMed PMID: 17915578; PubMed Central PMCID: PMC2949328].
[19] Stokes MB, Taylor AJ, McLean CA, D'Arcy CE, Mariani JA. Severe left ventricular hypertrophy and marked cardiac fibrosis in Danon disease. Int J Cardiol 2016;221:14–6. https://doi.org/10.1016/j.ijcard.2016.06.311 [Epub 2016 Jul1. PubMed PMID: 27395820].
[20] Yang Z, Vatta M. Danon disease as a cause of autophagic vacuolar myopathy. Congenit Heart Dis 2007;2(6):404–9. https://doi.org/10.1111/j.1747-0803.2007.00132.x [Review. PubMed PMID: 18377432].
[21] Butany Jagdish, Ibrahim Amir. LAMP2 mutation (Danon DISEASE). In: Miller DV, Revelo MP, editors. Diagnostic pathology cardiovascular. Manitoba: Amirsys Inc.; 2014. p. 1–20.
[22] Soliman OI, Timmermans RG, Nemes A, Vletter WB, Wilson JH, ten Cate FJ, et al. Cardiac abnormalities in adults with the attenuated form of mucopolysaccharidosis type I. J Inherit Metab Dis 2007;30(5):750–7 [Epub 2007 Jun 14. PubMed PMID: 17574537; PubMed Central PMCID: PMC2798132].
[23] Braunlin EA, Harmatz PR, Scarpa M, Furlanetto B, Kampmann C, Loehr JP, et al. Cardiac disease in patients with mucopolysaccharidosis: presentation, diagnosis and management. J Inherit Metab Dis 2011;34(6):1183–97. https://doi.org/10.1007/s10545-011-9359-8 [Epub 2011 Jul 9.Review. PubMed PMID: 21744090; PubMed Central PMCID: PMC3228957].
[24] Dangel JH. Cardiovascular changes in children with mucopolysaccharide storage diseases and related disorders — clinical and echocardiographic findings in 64 patients. Eur J Pediatr 1998;157:534–8.
[25] Fesslová V, Corti P, Sersale G, et al. The natural course and the impact of therapies of cardiac involvement in the mucopolysaccharidoses. Cardiol Young 2009;19:170–8.
[26] Tan CTT, Schaff HV, Miller FA, Edwards WD, Karnes PS. Valvular heart disease in four patients with Maroteaux–Lamy syndrome. Circulation 1992;85:188–95.
[27] Renteria VG, Ferrans VJ, Roberts WC. The heart in the Hurler syndrome. Gross, histologic and ultrastructural observations in five necropsy cases. Am J Cardiol 1976;38:487–501.
[28] Braunlin E, Orchard PJ, Whitley CB, Schroeder L, Reed RC, Manivel JC. Unexpected coronary artery findings in mucopolysaccharidosis. Report of four cases and literature review. Cardiovasc Pathol 2014;23(3):145–51. https://doi.org/10.1016/j.carpath.2014.01.001 [Epub 2014 Jan 10. Review. PubMed PMID: 24508139].
[29] Bolourchi M, Renella P, Wang RY. Aortic root dilatation in mucopolysaccharidosis I–VII. Int J Mol Sci 2016;17(12) [pii: E2004. PubMed PMID: 27916847; PubMed Central PMCID: PMC5187804].
[30] Hobbs JR, Hugh-Jones K, Barrett AJ, Byrom N, Chambers D, Henry K, et al. Reversal of clinical features of Hurler's disease and biochemical improvement after treatment by bone-marrow transplantation. Lancet 1981;2(8249):709–12 [PubMed PMID: 6116856].
[31] Penati R, Fumagalli F, Calbi V, Bernardo ME, Aiuti A. Gene therapy for lysosomal storage disorders: recent advances for metachromatic leukodystrophy and mucopolysaccaridosis I. J Inherit Metab Dis 2017;40(4):543–54. https://doi.org/10.1007/s10545-017-0052-4 [Epub 2017 May 30. Review. PubMed PMID: 28560469; PubMed Central PMCID: PMC5500670].
[32] Torloni MR, Franco K, Sass N. Gaucher's disease with myocardial involvement in pregnancy. Sao Paulo Med J 2002;120(3):90–2 [PubMed PMID: 12163901].
[33] Veinot JP, Elstein D, Hanania D, Abrahamov A, Srivatsa S, Zimran A. Gaucher's disease with valve calcification: possible role of Gaucher cells, bone matrix proteins and integrins. Can J Cardiol 1999;15(2):211–6 [PubMed PMID: 10079781].
[34] Kahn P. Anderson–Fabry disease: a histopathological study of three cases with observations on the mechanism of production of pain. J Neurol Neurosurg Psychiatry 1973;36(6):1053–62 [PubMed PMID: 4204059; PubMed Central PMCID: PMC1083608].

[35] Desnick RJ, Brady R, Barranger J, Collins AJ, Germain DP, Goldman M, et al. Fabry disease, an under-recognized multisystemic disorder: expert recommendations for diagnosis, management, and enzyme replacement therapy. Ann Intern Med 2003; 138(4):338–46 [Review. PubMed PMID: 12585833].
[36] Frustaci A, Morgante E, Russo MA, Scopelliti F, Grande C, Verardo R, et al. Pathology and function of conduction tissue in Fabry disease cardiomyopathy. Circ Arrhythm Electrophysiol 2015;8(4):799–805. https://doi.org/10.1161/CIRCEP.114.002569 [Epub 2015 Jun 5. PubMed PMID: 26047621].
[37] Putko BN, Wen K, Thompson RB, Mullen J, Shanks M, Yogasundaram H, et al. Anderson–Fabry cardiomyopathy: prevalence, pathophysiology, diagnosis and treatment. Heart Fail Rev 2015;20(2):179–91. https://doi.org/10.1007/s10741-014-9452-9 [Review. PubMed PMID: 25030479].
[38] Grunfeld JP, Lidove O, Joly D, Barbey F. Renal disease in Fabry patients. J Inherit Metab Dis 2001;24(Suppl. 2):71–4.
[39] Linhart A, Lubanda JC, Palecek T, Bultas J, Karetova D, Led- vinova J, et al. Cardiac manifestations in Fabry disease. J Inherit Metab Dis 2001;24(Suppl. 2):75–83.
[40] Tuttolomondo A, Pecoraro R, Simonetta I, Miceli S, Arnao V, Licata G, et al. Neurological complications of Anderson–Fabry disease. Curr Pharm Des 2013;19:6014–30.
[41] Shah JS, Elliott PM. Fabry disease and the heart: an overview of the natural history and the effect of enzyme replacement therapy. Acta Paediatr Suppl 2005;94(447): 11–4 [discussion 9-10. Review. PubMed PMID: 15895705].
[42] Perrot A, Osterziel KJ, Beck M, Dietz R, Kampmann C. Fabry disease: focus on cardiac manifestations and molecular mechanisms. Herz 2002;27(7):699–702 [PubMed PMID: 12439642].
[43] Yogasundaram H, Kim D, Oudit O, Thompson RB, Weidemann F, Oudit GY. Clinical features, diagnosis, and management of patients with Anderson–Fabry cardiomyopathy. Can J Cardiol 2017;33(7):883–97. https://doi.org/10.1016/j.cjca.2017.04.015 [Epub 2017 May 4. Review. PubMed PMID: 28668140].
[44] Eng CM, Germain DP, Banikazemi M, Warnock DG, Wanner C, Hopkin RJ, et al. Fabry disease: guidelines for the evaluation and management of multi-organ system involvement. Genet Med 2006;8(9):539–48 [Review. PubMed PMID: 16980809].
[45] Beck M, Hughes D, Kampmann C, Larroque S, Mehta A, Pintos-Morell G, et al. Long-term effectiveness of agalsidase alfa enzyme replacement in Fabry disease: a Fabry outcome survey analysis. Mol Genet Metab Rep 2015;3:21–7. https://doi.org/10.1016/j.ymgmr.2015.02.002 [eCollection 2015 Jun. PubMed PMID: 26937390; PubMed Central PMCID: PMC4750577].
[46] Yogasundaram H, Putko BN, Tien J, Paterson DI, Cujec B, Ringrose J, et al. Hydroxychloroquine-induced cardiomyopathy: case report, pathophysiology, diagnosis, and treatment. Can J Cardiol 2014;30(12):1706–15. https://doi.org/10.1016/j.cjca.2014.08.016 [Epub 2014 Aug 23. Review. PubMed PMID: 25475472].
[47] Dogar MU, Shah NN, Ishtiaq S, Shah PN, Shah P, Mathew S, et al. Hydroxychloroquine-induced restrictive cardiomyopathy: a case report. Postgrad Med J 2018;94(1109): 185–6. https://doi.org/10.1136/postgradmedj-2017-135236 [Epub 2018 Jan 20. PubMed PMID: 29353247].
[48] Arad M, Benson DW, Perez-Atayde AR, McKenna WJ, Sparks EA, Kanter RJ, et al. Constitutively active AMP kinase mutations cause glycogen storage disease mimicking hypertrophic cardiomyopathy. J Clin Invest 2002;109(3):357–62 [PubMed PMID: 11827995; PubMed Central PMCID: PMC150860].
[49] Banankhah P, Fishbein GA, Dota A, Ardehali R. Cardiac manifestations of PRKAG2 mutation. BMC Med Genet 2018;19(1):1. https://doi.org/10.1186/s12881-017-0512-6 [PubMed PMID: 29298659; PubMed Central PMCID: PMC5751825].
[50] Meyers DE, Basha HI, Koenig MK. Mitochondrial cardiomyopathy: pathophysiology, diagnosis, and management. Tex Heart Inst J 2013;40(4):385–94 [Review. PubMed PMID: 24082366; PubMed Central PMCID: PMC3783139].
[51] Arbustini E, Diegoli M, Fasani R, Grasso M, Morbini P, Banchieri N, et al. Mitochondrial DNA mutations and mitochondrial abnormalities in dilated cardiomyopathy. Am J Pathol 1998;153(5):1501–10 [PubMed PMID: 9811342;PubMed Central PMCID: PMC1853408].
[52] Marian AJ, Braunwald E. Hypertrophic cardiomyopathy: genetics, pathogenesis, clinical manifestations, diagnosis, and therapy. Circ Res 2017;121(7):749–70. https://doi.org/10.1161/CIRCRESAHA.117.311059 [. Review. PubMed PMID: 28912181; PubMed Central PMCID: PMC5654557].
[53] Ho CY. Hypertrophic cardiomyopathy: preclinical and early phenotype. J Cardiovasc Transl Res 2009;2(4):462–70. https://doi.org/10.1007/s12265-009-9124-7 [Epub 2009 Sep 26. Review. Erratum in: J Cardiovasc Transl Res. 2013 Aug;6(4):662. PubMed PMID: 20560004].
[54] Hughes SE. The pathology of hypertrophic cardiomyopathy. Histopathology 2004;44 (5):412–27 [Review. PubMed PMID: 15139989].
[55] Teekakirikul P, Kelly MA, Rehm HL, Lakdawala NK, Funke BH. Inherited cardiomyopathies: molecular genetics and clinical genetic testing in the postgenomic era. J Mol Diagn 2013;15(2):158–70. https://doi.org/10.1016/j.jmoldx.2012.09.002 [Epub 2012 Dec 27. Review. PubMed PMID: 23274168].
[56] Nishimura RA, Seggewiss H, Schaff HV. Hypertrophic obstructive cardiomyopathy: surgical myectomy and septal ablation. Circ Res 2017;121(7):771–83. https://doi.org/10.1161/CIRCRESAHA.116.309348 [Review. PubMed PMID: 28912182].
[57] Bekri S. Laboratory diagnosis of lysosomal storage diseases. In: Mehta A, Beck M, Sunder-Plassmann G, editors. Fabry disease: perspectives from 5 years of FOS. Oxford: Oxford PharmaGenesis; 2006 [Chapter 8].
[58] Meikle PJ, Ranieri E, Simonsen H, Rozaklis T, Ramsay SL, Whitfield PD, et al. Newborn screening for lysosomal storage disorders: clinical evaluation of a two-tier strategy. Pediatrics 2004;114:909–16.
[59] Humbel R, Collart M. Oligosaccharides in urine of patients with glycoprotein storage diseases. I. Rapid detection by thin-layer chromatography. Clin Chim Acta 1975;60: 143–5.
[60] Ramsay SL, Maire I, Bindloss C, Fuller M, Whitfield PD, Piraud M, et al. Determination of oligosaccharides and glycolipids in amniotic fluid by electrospray ionisation tandem mass spectrometry: *in utero*indicators of lysosomal storage diseases. Mol Genet Metab 2004;83:231–8.
[61] Conzelmann E, Sandhoff K. Partial enzyme deficiencies: residual activities and the development of neurological disorders. Dev Neurosci 1983;6:58–71.
[62] Taniguchi N, Kato E, Yoshida H, Iwaki S, Ohki T, Koizumi S. Alpha-glucosidase activity in human leucocytes: choice of lymphocytes for the diagnosis of Pompe's disease and the carrier state. Clin Chim Acta 1978;89:293e9.
[63] Shin YS, Endres W, Unterreithmeier J, Rieth M, Schaub J. Diagnosis of Pompe's disease using leukocyte preparations. Kinetic and immunological studies of 1,4-alpha-glucosidase in human fetal and adult tissues and cultured cells. Clin Chim Acta 1985;148:9e19.
[64] Umapathysivam K, Whittle AM, Ranieri E, Bindloss C, Ravenscroft EM, van Diggelen OP, et al. Determination of acid alpha-glucosidase protein: evaluation as a screening marker for Pompe disease and other lysosomal storage disorders. Clin Chem 2000; 46:1318e25.
[65] Filocamo M, Morrone A. Lysosomal storage disorders: molecular basis and laboratory testing. Hum Genomics. 2011;5(3):156–69 [Review. PubMed PMID: 21504867; PubMed Central PMCID: PMC3500170].
[66] Parenti G, Pignata C, Vajro P, Salerno M. New strategies for the treatment of lysosomal storage diseases (review). Int J Mol Med 2013;31(1):11–20. https://doi.org/10.3892/ijmm.2012.1187 [Epub 2012 Nov 19. Review. PubMed PMID: 23165354].
[67] Macauley SL. Combination therapies for lysosomal storage diseases: a complex answer to a simple problem. Pediatr Endocrinol Rev 2016;13(Suppl. 1):639–48 [Review. PubMed PMID: 27491211; PubMed Central PMCID: PMC5374980].