Eur J Pediatr (2005) 164: 509–514
DOI 10.1007/s00431-005-1678-z

ORIGINAL PAPER

**Christian Balmer · Diana Ballhausen · Nils U. Bosshard
Beat Steinmann · Eugen Boltshauser · Urs Bauersfeld
Andrea Superti-Furga**

Familial X-linked cardiomyopathy (Danon disease): diagnostic confirmation by mutation analysis of the *LAMP2*gene

Received: 4 January 2005 / Accepted: 9 March 2005 / Published online: 12 May 2005

Abstract A boy presented at age 2.5 years with mild left ventricular hypertrophy and mild myopathy. Hypertrophic cardiomyopathy progressed relentlessly, leading to death at age 16 years shortly before planned heart transplantation. During the course of the disease, his mother developed severe dilated cardiomyopathy and died of its complications at 46 years of age. The combination of myopathy and cardiomyopathy, the biochemical and electron microscopy findings in a muscle biopsy, and the pedigree suggested Danon disease (MIM 300257), an X-linked lysosomal storage disorder caused by deficiency of lysosome-associated membrane protein-2 (LAMP2). The diagnosis was confirmed by the identification of a novel mutation, G138A, in the *LAMP2*gene, leading to the premature stop codon W46X. *Conclusion*: Early diagnosis of Danon disease is important for genetic counselling and timely cardiac transplantation, the only effective therapeutic option.

Keywords Danon disease · Glycogen storage disease with normal acid maltase · LAMP2 deficiency · Lysosomal membrane · X-linked vacuolar cardiomyopathy and myopathy

Abbreviations *LAMP2:* lysosome-associated membrane protein-2 · *SSCP:* single strand conformational polymorphism

C. Balmer (✉) · U. Bauersfeld
Department of Paediatric Cardiology,
University Children's Hospital,
Steinwiesstrasse 75, 8032 Zurich, Switzerland
E-mail: christian.balmer@kispi.unizh.ch
Tel.: +41-1-2667111
Fax: +41-1-2667981

D. Ballhausen · N. U. Bosshard · B. Steinmann
Division of Metabolism and Molecular Paediatrics,
University Children's Hospital,
Zurich, Switzerland

E. Boltshauser
Division of Neurology, University Children's Hospital,
Zurich, Switzerland

A. Superti-Furga
Division of Molecular Paediatrics,
University Hospital Lausanne (CHUV),
Lausanne, Switzerland

Introduction

Danon disease (MIM 300257) is a rare X-linked disorder characterised by cardiomyopathy, skeletal myopathy, and mental retardation. The myopathy is generally mild and the mental retardation variable, but cardiomyopathy dominates the clinical picture and determines the outcome. Mutations in the *LAMP2*-gene on Xq24 have been shown to be responsible for the disease, the cellular pathogenesis being caused by a deficiency of lysosome-associated membrane protein-2 (LAMP2). This explains the lysosomal storage that occurs in this disorder, which includes elevated muscle glycogen in spite of normal α-glucosidase (acid maltase) activity. The pathological hallmark of the disease is cytoplasmic vacuoles containing autophagic material and glycogen in skeletal and cardiac muscle cells. Understanding of Danon disease in man has been aided by the availability of an animal model, the lamp2-deficient mouse; lamp2-deficient mice have reduced weight and increased mortality, and autophagic vacuoles accumulate in many tissues including skeletal and heart muscle, liver, pancreas, spleen, and kidney. In hepatocytes, the autophagic degradation of long-lived proteins is severely impaired, cardiac myocytes are ultrastructurally abnormal and heart contractility is severely reduced [19,23].

This report of a family with Danon disease demonstrates the typical course of the disease and the difficulty of determining the right moment for cardiac transplantation.

Case report

The mother of the patient suffered from severe dilated cardiomyopathy with atrial fibrillation. The onset of symptoms was at age 37 years and an endomyocardial biopsy showed intracellular vacuoles which were frequently located near the nuclei. A granular pigment was also scattered over the whole specimen. Light microscopy showed localised focal fibrosis of the endocardium and widespread myocardial necrosis of both ventricles. There was marked coronary sclerosis. The mother died at age 46 years, during evaluation for cardiac transplantation, due to intimal dissection of the right coronary artery on cardiac catheterisation. Autopsy was not done and a diagnosis of Danon disease was not suspected during her lifetime. The maternal family is of Swiss origin. Family history showed that the mother had an older brother and a younger sister who are both healthy, and did not indicate further affected individuals.

The patient, who had an older sister and a younger brother, both of whom are healthy, was born at term after a normal pregnancy. Fetal movements were reported to be normal. Neonatal adaptation and gross motor development were also normal. At age 2.5 years the patient presented with mild tiredness and attacks of abdominal pain. He also had difficulty standing up after falls. Abdominal symptoms disappeared after some months but the mild exercise intolerance (NYHA II) persisted. Clinical examination of the heart was normal. Initial ECG was normal and echocardiography at age 3 years showed only mild thickening of the left ventricular posterior wall. Serum creatine kinase was elevated at 943 U/l (normal < 196 U/l).

He received speech and language therapy for a moderate delay in expressive speech development at age 7 years. There were also mild learning disabilities with an average performance at primary school.

Echocardiography over time showed a continuous thickening of the myocardial wall, which resulted in a severe concentric hypertrophy of both ventricles without obstruction of the left ventricular outflow tract (Fig. 1). Chest radiography showed severe cardiomegaly (Fig. 2).

At age 10 years he complained of recurrent palpitations that were controlled with atenolol. ECG at age 11 years demonstrated pre-excitation with a left bundle branch block pattern (Fig. 3). Holter monitoring showed runs of nonsustained AV re-entrant tachycardia.

Physical examination 15 years of age revealed a prominent left ventricular impulse and a grade 3 ejection systolic murmur at the left sternal border. Neurological examination was normal. Congestive heart failure progressed rapidly at age 16 years and the patient suffered from recurrent fainting. A left ventricular assist device was implanted as a bridge to transplantation [9]. Unfortunately, during the 4th month subsequent to this, the patient died of sepsis. Consent for an autopsy was not given.

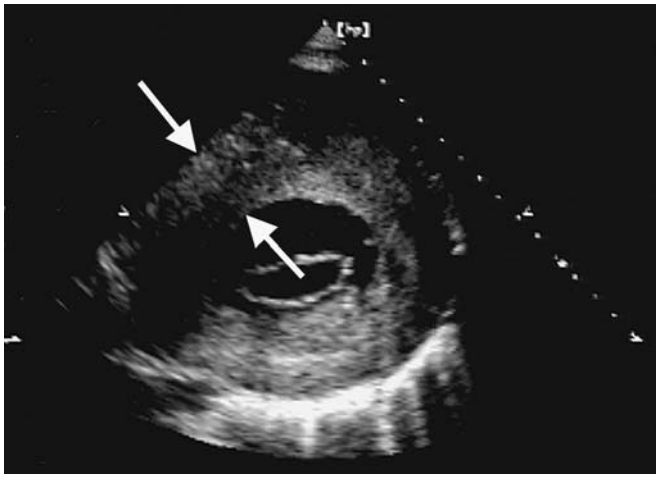


Fig. 1 Echocardiography at age 11 years. Parasternal short axis view showing severe concentric hypertrophy of the left ventricle (*arrows*)

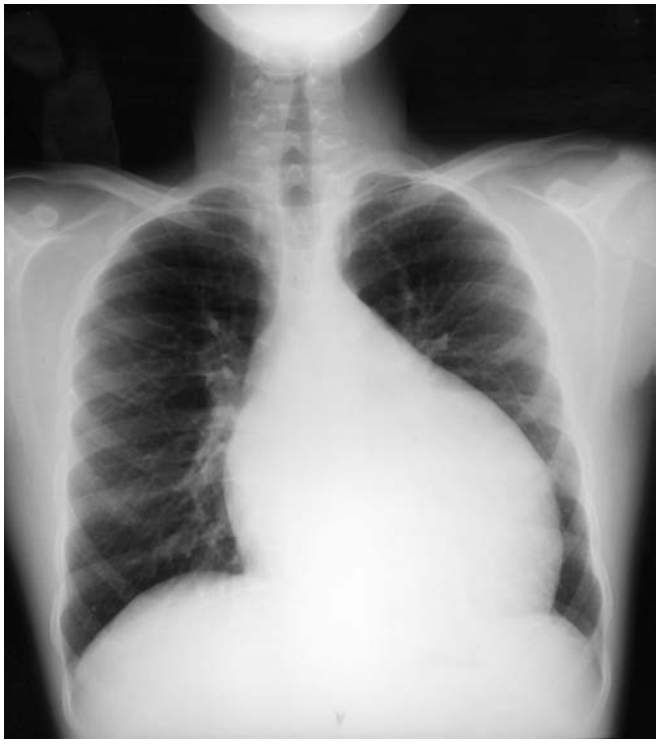


Fig. 2 Chest X-ray film taken at age 14 years showing severe cardiomegaly

Materials and Methods

Enzyme assays

In muscle, glycogen content and activities of α-glucosidase (EC 3.2.1.20) and phosphorylase (EC 2.4.1.1) were determined according to Hers [14]. The activities

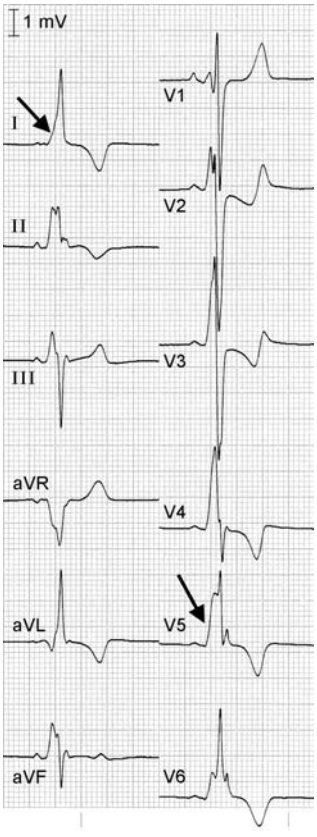


Fig. 3 12-lead ECG obtained at age 11 years. Sinus rhythm with signs of severe left ventricular hypertrophy, left bundle branch block, and delta wave (arrows)

of phosphofructokinase (EC 2.7.1.11) and phosphorylase b-kinase (EC 2.7.1.38) were determined according to Beutler [4] and Huijing [16], respectively, and the mean outer chain length of glycogen was determined by a glycogen-iodine-spectrum as described by Kriss-man [17]. In serum, leukocytes, and fibroblasts, activities of the lysosomal enzymes α-iduronidase (EC 3.2.1.76), α-fucosidase (EC 3.2.1.51), α-galactosidase (EC 3.2.1.22), β-galactosidase (EC 3.2.1.23), β-glucosidase (EC 3.2.1.21), β-glucuronidase (EC 3.2.1.31), and β-hexosaminidase (EC 3.2.1.50) were determined fluorimetrically according to Galjaard [12], while acid phosphatase (EC 3.1.3.2) and arylsulfatase A (EC 3.1.6.8) activities were determined according to Beutler et al. [5] and Baum et al. [2], respectively.

Molecular analysis

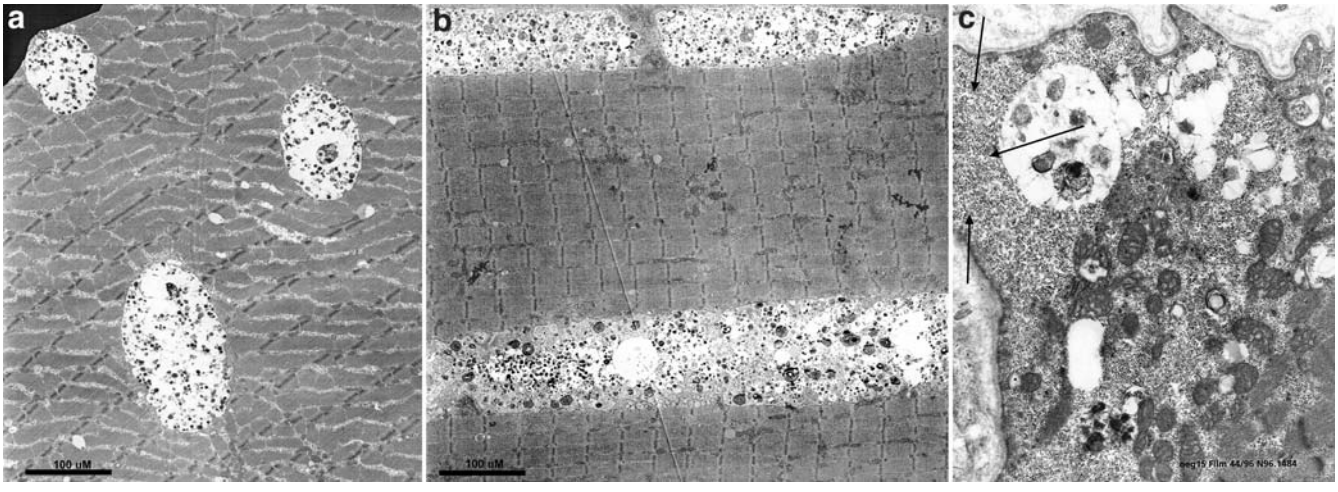
Primers were designed for the amplification of exons 1–8, 9a and 9b of *LAMP2* (chromosome Xq24) [11]. PCR products were subjected to single strand conformational polymorphism (SSCP) analysis following standard protocols. For exons showing a band shift in SSCP, direct sequencing was performed using an ABI Prism 310 sequencer.

Results

Morphological findings

Skeletal muscle biopsy of the vastus lateralis muscle was done at age 11 years. Light microscopy revealed central vacuoles with irregular margins. Only a small proportion of muscle fibres were atrophic. One region contained inflammatory infiltrates. Immunohistochemical staining of the central vacuoles was positive for laminin, dystrophin-associated glycoprotein, and dystrophin. Electron microscopy demonstrated large amounts of free glycogen between the myofibrillar bundles and subsarcolemmally. Glycogen was also present in lysosomes. Numerous cytoplasmic, cylinder-shaped structures were present, their major axes being oriented along the myofibrils (Fig. 4). Their content was highly polymorphic. These structures are typical of Danon disease and are believed to represent autophagic vacuoles.

Fig. 4 Skeletal muscle: (**a**) autophagic vacuoles (EM ×3150); (**b**) a different orientation reveals the tubular shape of the autophagic vacuoles (EM ×3150); (**c**) Excessive and diffuse accumulation of glycogen visible as fine granules (*arrows*) (EM ×12500)



Biochemical findings

Muscle glycogen content was slightly elevated (2.8%, reference range 0.7%–2.0%) but the glycogen outer chain length was normal. Normal activity of α-glucosidase, phosphorylase, phosphofructokinase, phosphorylase b-kinase, and the normal outer chain length of glycogen excluded glycogen storage diseases II (Pompe disease), III (debranching enzyme deficiency), V (McArdle disease), VII (Tarui disease), and IX (phosphorylase b-kinase deficiency).

The following enzymes were consistently elevated in serum, the values given being those at age 11 years: creatine kinase 1521 U/l (normal < 196 U/l), creatine kinase isoenzyme MB 28 U/l (< 25 U/l), alkaline phosphatase 265 U/l (< 105 U/l), aspartate aminotransferase 378 U/l (< 29 U/l), alanine aminotransferase 413 U/l (< 27 U/l), and lactate dehydrogenase 4158 U/l (< 657 U/l).

At age 15 years, several acid hydrolases were measured in serum and leukocyte homogenate: activities of α-fucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, and β-hexosaminidases A and B were within the respective reference ranges in serum and near the lower ends of the reference ranges in leukocytes. In cultured skin fibroblasts, acid phosphatase, arylsulfatase A, α-fucosidase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, β-glucuronidase, β-hexosaminidase A + B, and α-iduronidase activities were all within the respective reference ranges.

Molecular findings

SSCP analysis showed a band shift in the PCR product of *LAMP2*exon 2 from the patient (Fig. 5). Subsequent direct sequencing on an ABI Prism 310 sequencer detected a 138 G → A point mutation (W46X) which resulted in a premature stop codon being introduced at codon 46. Assuming that mRNA containing the premature stop codon escaped nonsense-mediated decay, it would direct the synthesis of a truncated LAMP2 protein lacking a major part of the carboxyl terminal domain, including the whole transmembrane region. The mutation was not present in the patient's healthy sister and brother. There was no maternal material available for investigation.

Discussion

The characteristics of Danon disease have been described in a number of case reports [3, 6, 7, 8, 10, 13, 21, 24, 25,26]. The typical clinical picture includes childhood onset, male predominance (1.8:1) [26], cardiomyopathy, mild myopathy, and mental retardation of variable degree. Cardiomyopathy in affected males is usually hypertrophic with childhood onset [20]. Its course is progressive and is the cause of death from arrhythmia or severe heart failure, usually at 18 to 19 years of age [20,26]. Affected female relatives appear to have less severe cardiomyopathy with mainly dilative morphology and later onset of symptoms, and death is usually later, at 37 to 40 years of age [20,26]. ECG may be normal in the early stages of the disease but is pathological in all patients at a later stage. The most frequent finding is Wolff-Parkinson-White syndrome, which is believed to be due to a disruption of the annulus fibrosus by glycogen-engorged myocytes, as demonstrated in an animal model for constitutional activation of PRKAG2, another condition with pre-excitation due to glycogen storage within cardiomyocytes [1].

Muscle biopsy shows a characteristic vacuolar myopathy with PAS-positive inclusions in heart and skeletal muscle myocytes. Electron micrographs show large amounts of glycogen distorting the myofibrillar bundles, lysosomes filled with glycogen, and numerous cylinder-shaped vacuoles full of partially degraded

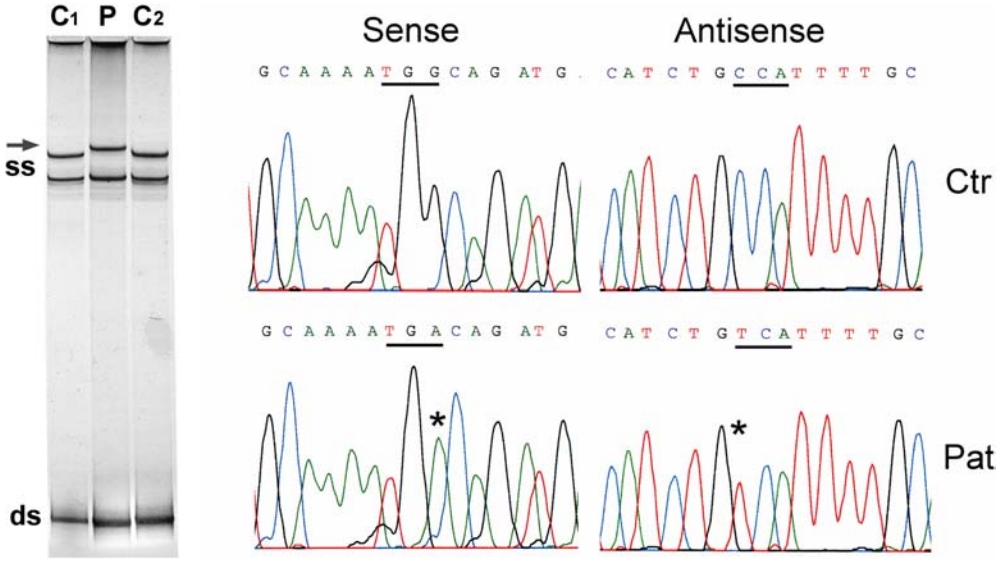


Fig. 5 Left panel: SSCP pattern of exon 2 of *LAMP2* amplified from two control DNA samples (C1 and C2) and the patient's DNA (P). ds indicates double-stranded and ss single-stranded PCR products. Note mobility shift in the ss population from the patient. Right panel: bidirectional sequence analysis of exon 2 in *LAMP2* showing hemizygosity for a G → A transition at position 138 leading to the change of codon 46 from TGG (Trp) to TGA (stop)

autophagic material. These latter findings are characteristic of Danon disease. Myocardial fibrosis may occur in the course of the disease. The accumulation of glycogen can be confirmed biochemically and has, in the past, led to the classification of Danon disease as a variant form of Pompe disease (GSD II) with lysosomal glycogen accumulation but normal α-glucosidase [8].

The biochemical findings are in contrast to those found in mucolipidosis II/III, another generalised lysosomal disorder, in which the activities of several lysosomal enzymes are drastically increased in serum while being reduced in fibroblasts. Therefore, Danon disease cannot be diagnosed biochemically by measuring these enzymes in serum.

The genetic defect has been localised to Xq24, and some 25 mutations have been identified to date [15, 18, 22,26]. The *LAMP2*gene encodes LAMP2 which has a transmembrane domain believed to project from the lysosomal membrane into the lysosomal space. The absence of LAMP2 leads to an accumulation of autophagic vacuoles, mainly in heart and skeletal muscle cells, but also in other tissues, e.g. liver and platelets [20]. Most mutations lead to premature truncation of LAMP2 with abolition of the transmembrane domain (as in our case), and thus can be assumed to represent null alleles. The more severe "neonatal form" of glycogen storage disease with normal acid maltase is most probably caused by mutations in a different gene [26]. At present, Danon disease is the only example of cardiomyopathy and skeletal myopathy caused by a mutation in a lysosomal structural protein rather than a lysosomal enzyme.

Unfortunately, there is no causal therapy for Danon disease. The therapeutic aim for patients is to prevent progressive heart failure and to reduce the risk of arrhythmias and sudden death. As the disease progresses, the only remaining therapeutic option is cardiac transplantation. Since mental retardation and skeletal myopathy are not progressive, the prognosis after transplantation is good [10]. The timing of cardiac transplantation still remains difficult, as exemplified by this patient. With the advent of mutation analysis it is possible to confirm the diagnosis, plan a therapeutic regime, and offer genetic counselling.

Acknowledgement We thank Dr. Max A. Spycher, Department of Pathology, Faculty of Medicine, University of Zurich, for his excellent electron microscopic evaluation.

References

1. Arad M, Moskowitz IP, Patel VV, Ahmad F, Perez-Atayde AR, Sawyer DB, Walter M, Li GH, Burgon PG, Maguire CT, Stapleton D, Schmitt JP, Guo XX, Pizard A, Kupershmidt S, Roden DM, Berul CI, Seidman CE, Sedman JG (2003) Transgenic mice overexpressing mutant PRKAG2 define the cause of Wolff-Parkinson-White syndrome in glycogen storage cardiomyopathy. Circulation 107: 2850–2856
2. Baum H, Dodgson KS, Spencer B (1959) The assay of arylsulphatases A and B in human urine. Clin Chim Acta 4: 453–455
3. Bergia B, Sybers HD, Butler IJ (1986) Familial lethal cardiomyopathy with mental retardation and scapuloperoneal muscle dystrophy. J Neurol Neurosurg Psychiatry 49: 1423–1426
4. Beutler E (1975) Red cell metabolism. Grune and Stratton, New York, pp 42–45
5. Beutler E, Kuhl W, Matsumoto F, Pangalis G (1976) Acid hydrolases in leukocytes and platelets of normal subjects and in patients with Gaucher's and Fabry's disease. J Exp Med 143: 975–980
6. Bru P, Pellissier JF, Gatau-Pelanchon J, Faugère G, de Barsy T, Levy S, Gérard R (1988) Glycogenose lysosomiale cardiomusculaire de l'adulte sans deficit enzymatique connu. Arch Mal Coeur Vaiss 81: 109–114
7. Byrne E, Dennett X, Crotty B, Trounce I, Sands JM, Hawkins R, Hammond J, Anderson S, Haan EA, Pollard A (1986) Dominantly inherited cardioskeletal myopathy with lysosomal glycogen storage and normal acid maltase levels. Brain 109: 523–536
8. Danon MJ, Oh SJ, DiMauro S, Manaligod JR, Eastwood A, Naidu S, Schliselfeld LH (1981) Lysosomal glycogen storage disease with normal acid maltase. Neurology 31: 51–57
9. DeBakey ME, Bernowski R (1998) The DeBakey/NASA axial flow ventricular assist device. In: Akutsu T, Koyanagi H (eds) Heart replacement and artificial heart. Springer, Tokyo, pp 407–413
10. Dworzak F, Casazza F, Mora M, De Maria R, Gronda E, Baroldi G, Rimoldi M, Morandi L, Cornelio F (1994) Lysosomal glycogen storage with normal acid maltase: a familial study with successful heart transplant. Neuromuscul Disord 4: 243–247
11. Fukuda M (1994) Biogenesis of the lysosomal membrane. Subcell Biochem 22: 199–230
12. Galjaard H (1980) Genetic metabolic diseases. Early diagnosis and prenatal analysis. Elsevier, Amsterdam, pp 812–828
13. Hart ZH, Servidei S, Peterson PL, Chang CH, DiMauro S (1987) Cardiomyopathy, mental retardation and autophagic vacuolar myopathy. Neurology 37: 1065–1068
14. Hers HG (1964) Glycogen storage disease. In: Levine R, Luft R (eds) Advances in metabolic disorders, vol. I. Academic Press, New York London, pp 1–44
15. Horvath J, Ketelsen UP, Geibel-Zehender A, Boehm N, Olbrich H, Korinthenberg R, Omran H (2003) Identification of a novel LAMP2 mutation responsible for X-chromosomal dominant Danon disease. Neuropediatrics 34: 270–273
16. Huijing F (1974) Glycogen and enzymes of glycogen metabolism. In: Curtius HC, Roth M (eds) Clinical biochemistry, vol. II. W. de Gruyter, Berlin New York, pp 1208–1235
17. Krisman CR (1962) A method for the colorimetric estimation of glycogen with iodine. Anal Biochem 4: 17–23
18. Nishino I, Fu J, Tanji K, Yamada T, Shimojo S, Koori T, Mora M, Riggs JE, Oh SJ, Koga Y, Sue CM, Yamamoto A, Murakami N, Shanske S, Byrne E, Bonilla E, Nonaka I, DiMauro S, Hirano M (2000) Primary LAMP-2 deficiency causes X-linked vacuolar cardiomyopathy and myopathy (Danon disease). Nature 406: 906–910
19. Saftig P, Tanaka Y, Lüllmann-Rauch R, von Figura K (2001) Disease model: LAMP-2 enlightens Danon disease. Trends Mol Med 7: 37–39
20. Sugie K, Yamamoto A, Murayama K, Oh SJ, Takahashi M, Mora M, Riggs JE, Colomer J, Iturriaga C, Meloni A, Lamperti C, Saitoh S, Byrne E, DiMauro S, Nonaka I, Hirano M, Nishino I (2002) Clinicopathological features of genetically confirmed Danon disease. Neurology 58: 1773–1778
21. Tachi N, Tachi M, Sasaki K, Tomita H, Wakai S, Annaka S, Minami R, Tsurui S, Sugie H (1989) Glycogen storage disease with normal acid maltase: skeletal and cardiac muscles. Pediatr Neurol 5: 60–63

22. Takahashi M, Yamamoto A, Takano K, Sudo A, Wada T, Goto Y, Nishino I, Saitoh S (2002) Germline mosaicism of a novel mutation in lysosome-associated membrane protein-2 deficiency (Danon disease). Ann Neurol 52: 122–125
23. Tanaka Y, Guhde G, Suter A, Eskelinen EL, Hartmann D, Lüllmann-Rauch R, Janssen PM, Blanz J, von Figura K, Saftig P (2000) Accumulation of autophagic vacuoles and cardiomyopathy in LAMP-2-deficient mice. Nature 406: 902–906
24. Tse HF, Shek TW, Tai YT, Lau YK, Ma L (1996) Case report: lysosomal glycogen storage disease with normal acid maltase: an unusual form of hypertrophic cardiomyopathy with rapidly progressive heart failure. Am J Med Sci 312: 182–186
25. Usuki F, Takenega S, Higuchi I, Kashio N, Nakagawa M, Osame M (1994) Morphologic findings in biopsied skeletal muscle and cultured fibroblasts from a female patient with Danon's disease (lysosomal glycogen storage disease without acid maltase deficiency). J Neurol Sci 127: 54–60
26. Verloes A, Massin M, Lombet J, Grattagliano B, Soyeur D, Rigo J, Koulischer L, Van Hoof F (1997) Nosology of lysosomal glycogen storage diseases without in vitro acid maltase deficiency. Delineation of a neonatal form. Am J Med Genet 72: 135–142