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# A new phenotype of severe dilated cardiomyopathy associated with a mutation in the *LAMP2* gene previously known to cause hypertrophic cardiomyopathy in the context of Danon disease

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ABSTRACT

Danon disease is a rare X-linked cardiac and skeletal muscle disorder with multisystem clinical manifestations. Genetic defects at the lysosome-associated membrane 2 protein (LAMP2) are the cause of the disorder. Due to the rarity of the disease, there is limited progress in understanding the correlation between genotype and phenotype, and explaining the large variability of the clinical features of the disease. In this study, we report two patients, twin sisters, referred to our hospital for end stage heart failure due to dilated cardiomyopathy, requiring heart transplant evaluation. Genetic analysis, using targeted next generation sequencing, showed that the proband carried a *LAMP2* missense variant, c.928G > A. The mutation was also detected in her twin sister by sanger sequencing. This variant has already been reported by other investigators and was correlated with the clinical triad of Danon disease i.e. hypertrophic cardiomyopathy, mental retardation and peripheral myopathy. The new phenotype of dilated cardiomyopathy associated with this mutation, confirms the phenotypic heterogeneity of the particular mutation, as well as of Danon disease.

## 1. Introduction

Danon disease is a rare X-linked dominant cardioskeletal myopathy, caused by mutations in the Lysosome-Associated Membrane Protein-2 gene (*LAMP-2*), that in turn cause an abnormal expression of the LAMP2 protein, which is a component of the lysosomal membrane. This deficiency is associated with the presence of cytoplasmic vacuolation of the myocytes which contain autophagic material including glycogen (Endo et al., 2015). The X-linked nature of inheritance accounts for reported differences in phenotypic severity between males and females. Classical clinical features in males include hypetrophic cardiomyopathy (HCM), skeletal myopathy and mental retardation (Cheng and Fang, 2012; Danon et al., 1981), while female carriers show milder and later onset symptoms of cardiomyopathy (approximately equal prevalence of dilated and hypertrophic), rarely associated with myopathy (Bertini et al., 2005; Boucek et al., 2011; D' Souza et al., 2014). Other manifestations of Danon disease include Wolff-Parkinson-White (WPW) syndrome, increased serum creatine kinase (CK) and ophthalmic abnormalities (Prall et al., 2006). Due to the heterogeneity and rarity of the disease, the complete phenotype is not well understood and the diagnostic approach is often difficult. Many mutations in *LAMP2* gene have been described in association with Danon disease, but there is no clear correlation between genotype and phenotype yet (Boucek et al., 2011; Fu et al., 2016; Sabourdy et al., 2009). In the present study we report two patients that are twin sisters, both carrying a missense mutation in the *LAMP2* gene, c.928G > A, which is the most frequent mutation reported in this gene (D'souza et al., 2014). So far, this mutation has been described in male patients presented with hypertrophic cardiomyopathy (Arad et al., 2005), with classical Danon disease of mild myopathy combined with HCM (Bertini et al., 2005; Maron et al., 2009; Sabourdy et al., 2009) and in one case with voltage hypertrophy of the ventricles, motor delay and autism (Burusnukul et al., 2008). In the present report both females manifested severe dilated cardiomyopathy (DCM) and underwent heart transplantation.

## 2. Clinical report

### 2.1. Patient 1

The first patient is a 36 years old female, referred to our hospital for advanced heart failure treatment, requiring urgent heart transplant evaluation. Dilated cardiomyopathy was diagnosed a year ago in the Emergency Department of the Local Hospital with new onset atrial fibrillation with rapid ventricular response and signs of acute heart failure. Her father died at 38 years of age and according to the descriptions given from the family environment, he suffered from multiorgan failure due to end stage heart failure from unknown reason. Her mother is healthy at 59 years of age (Fig. 1a).

During the heart transplant evaluation, serial echocardiographic assessments revealed typical images of dilated cardiomyopathy. Left ventricle was dilated with spherical configuration of its cavity [sphericity index (SI: 1.09)], uniform thinning (8 mm) and diffuse severe hypokinesis of its walls along with a severe impairment of its systolic function. Left ventricular end diastolic diameter (LVEDd) was 66 mm, end systolic diameter (ESD) 56 mm and ejection fraction (LVEF) 25%. Right ventricular function was initially marginally preserved, but deteriorated over the next few months. Pulmonary hypertension was detected (RVSP: 58 mmHg). 24 hour Holter ECG monitoring revealed intraventricular conduction abnormalities demonstrated as alternating right and left bundle branch block (LBBB and RBBB). This finding along with the severely impaired LVEF led to the implantation of an implantable defibrillator (ICD). Cardiopulmonary exercise testing revealed severe deterioration of her functional status with a peak $VO_2$ of 10.7 mL/kg/min. Right heart catheterization was performed with the following findings: PA: 41/22/30 mmHg, PCWP: 19 mmHg, transpulmonary gradient (TPG) of

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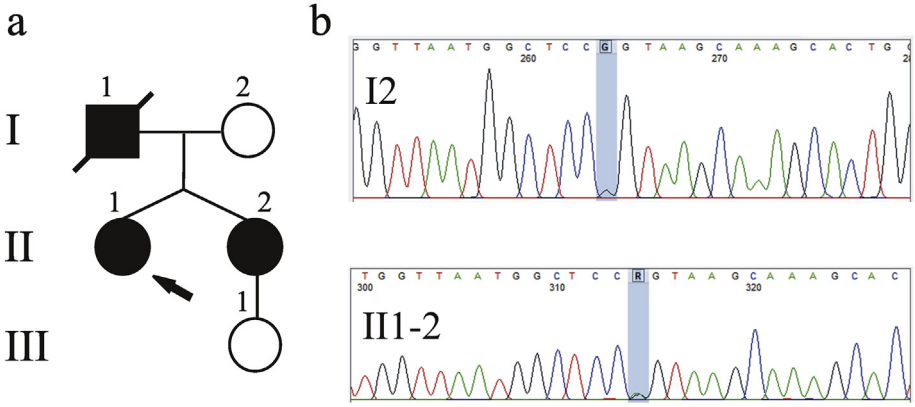


**Fig. 1. a)** Pedigree of the two affected sisters. **b)** Electropherogram of the involved sequence fragment of *LAMP2* for the members of the family, the result of forward sequence is shown. The wild type and the detected mutation are highlighted. The mutation is present in both sisters (II 1, 2) but not in their mother (I2).

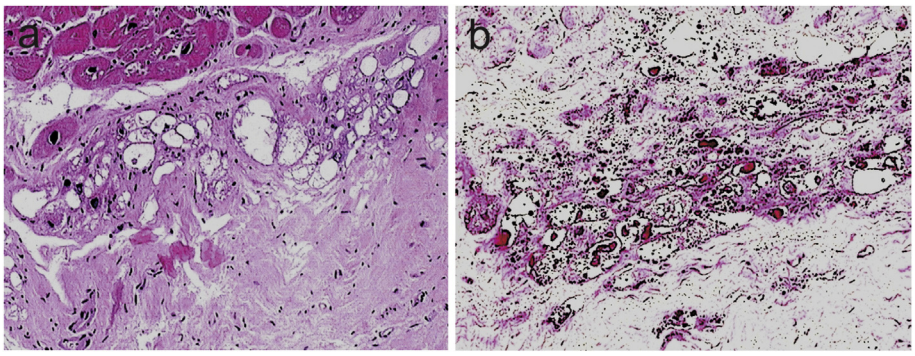


**Fig. 2.** Histopathology of the heart from the proband. **a)** Paraffin sections stained with hematoxylin-eosin (×100). Diffuse interstitial fibrosis and hypertrophic myocytes with vacuolated cytoplasm, resembling rabdomyoma cells, due to accumulation of material including glycogen **b)** Paraffin sections stained with Periodic acid Schiff (×20). The storaged PAS-positive material is depicted red in the cytoplasm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

11 mmHg and cardiac output (CO) of 2.6 L/min.

Laboratory testing revealed elevated bilirubin at 6.3 mg/dL (direct bilirubin 3.34 mg/dL) with relatively normal values of AST at 64 IU/L, ALT at 27 IU/L, γGT at 46 IU/L and ALP at 76 IU/L. The bilirubin value was attributed to her severe biventricular heart failure. LDH levels were also elevated at 903 IU/L and CK levels were within normal range. She also had an impaired glucose tolerance test (0 min: 71.6 mg/dL, 60 min: 197mg/dL, 120 min: 191mg/dL). The neurological evaluation revealed no pathological findings. Imaging testing revealed an enlarged liver (longitudinal diameter at 18 cm as measured in the abdominal CT) with heterogeneous parenchyma. Myocardial biopsy showed marked deposition of PAS-positive material in the cytoplasm of the myocytes and diffuse interstitial fibrosis (Fig. 2). The patient was included to the national heart transplantation waiting list and an uneventful heart transplantation was performed after 12 months.

### 2.2. Patient 2

First patient's twin sister was urgently referred to our hospital due to pulmonary oedema at 38 years of age. In the emergency department atrial fibrillation with rapid ventricular response and subsequent hemodynamic instability required urgent electrical cardioversion. Right heart catheterization revealed right atrial pressure of 5 mmHg, right ventricular (RV) systolic pressure of 44 mmHg, RV diastolic pressure of 4 mmHg, systolic pulmonary artery (PA) pressure of 44 mmHg, diastolic PA pressure of 25 mmHg, mean PA pressure of 32 mmHg, pulmonary capillary wedge pressure (PCWP) of 23 mmHg and transpulmonary gradient (TPG) of 9 mmHg.

Echocardiography also revealed a severely dilated and distorted left ventricle (EDD 69 mm, ESD 61 mm, SI: 1.05) with thin walls (6 mm) and severely impaired systolic function (LVEF: 20%) and subsequent pulmonary hypertension. No ventricular arrhythmias were noted. Cardiopulmonary exercise testing (peak $VO_2$ of 13.2 mL/kg/min) was compatible with stage D functional status (Weber Classification). Laboratory tests revealed impaired liver function (AST: 123 IU/L, ALT: 163 IU/L, LDH at 404 IU/L). γGT, ALP and CK levels were within normal range, while bilirubin levels were at 2.5 mg/dL (direct bilirubin 0.57 mg/dL). She also had an impaired glucose tolerance test (0 min: 95.1 mg/dL, 60 min: 224.3 mg/dL, 120 min: 137.8 mg/dL).

Imaging testing revealed an enlarged liver. Endomyocardial biopsy showed diffuse interstitial fibrosis and groups of myocytes, most of which showed vacuolation of the cytoplasm, similarly to that observed in the biopsy of her sister. Neurological evaluation was conducted by two independent neurologists without identifying any pathological findings.

The molecular basis of the disease was identified with next generation sequencing (NGS) technology, using Illumina's Trusight Cardio sequencing panel, covering 174 genes clinically relevant to cardiac diseases. Written informed consent for molecular genetic testing was obtained from both patients and their mother, and the study was approved by the Ethics Committee of the Onassis Cardiac Surgery Center. Genetic testing detected a pathogenic mutation in *LAMP2* gene (NM_001122606.1: c.928G > A), rs104894858, that is strongly related to Danon disease. The mutation was confirmed by Sanger sequencing for both sisters, but was absent from their mother (Fig. 1b).

## 3. Discussion

In this study we report two twin sisters carrying the same *LAMP2* gene mutation and manifesting dilated cardiomyopathy, as opposed to the phenotype of HCM described previously in the literature for the carriers of this particular mutation. Another *LAMP2* mutation has been observed once before in monozygotic twins (Hashida et al., 2015, Yoshida et al., 2018) but unlike our case, the two 18 years-old girls displayed different phenotypic features.

The *LAMP2* gene mutation that was detected in our patients,

**Table 1**
**Clinical features of patients harboring the *LAMP2* mutation 928G > A.** M: male; F: female; DCM: dilated cardiomyopathy; HCM: hypertrophic cardiomyopathy; LV: left ventricular; ECG: electrocardiogram; LVEF: left ventricular ejection fragment; AST: aspartate aminotransferase; ALT: alanine aminotransferase; γGT: gamma-glutamyl transferase; LDH: lactic acid dehydrogenase; CK: creatine kinase.

| Patients | Sex | Age at diagnosis | Echocardiography | LVEF (%) | Relevant lab values (IU/L) | Clinical symptoms |
|---|---|---|---|---|---|---|
| Patient 1 (this study) | F | 36years | DCM | 25 | AST: 64, ALT: 27, γGT: 46, LDH: 903, CK: normal range | No neurological/myoskeletal findings, no mental retardation |
| Patient 2 (this study) | F | 38years | DCM | 20 | AST: 123, ALT: 163, γGT: normal, LDH: 404, CK: normal range | Atrial fibrillation, no neurological/myoskeletal findings, no mental retardation |
| Family LS, II2 (Arad et al., 2005) | M | | HCM | 60 | ALT (2× normal values), CK (2× normal values) | No mental retardation, no neurologic/myoskeletal deficits |
| Family LO, II1 (Arad et al., 2005) | M | | HCM | 60 | ALT (2× normal values), CK (2× normal values) | No mental retardation, no neurologic/myoskeletal deficits |
| Patient 1 (Bertini et al., 2005) | M | 4months | Severe obstructive HCM | | CK (2–3 x normal values) | Hypotonia, signs of cardiac failure, WPW syndrome in the following years |
| Patient 2 (Bertini et al., 2005) | M | 18months | HCM | | AST: 288, ALT: 344, γGT: normal, LDH: 188, CK: 835 | Moderate delay in motor milestones, mild mental retardation, delay in language development, diffuse muscle weakness at the age of 15 years |
| Patient A (Sabourdy et al., 2009) | M | 13months | No LV hypetrophy, ECG: signs of hypetrophy | 56 | AST: 315, ALT: 419, LDH: 964, CK: 700-900 | Moving difficulties at infancy, mild muscular hypotonia and mild learning and speaking problems at the age of 4 years |
| Burusnukul et al., 2008 | M | 16months | No LV hypetrophy, ECG: signs of hypetrophy | | AST: 294, ALT: 144, CK: 1202 | Motor delay, diffuse hypotonia, severe autism |

c.928G > A, corresponds to an isoleucine for valine substitution at residue 310, but is also the very last nucleotide of exon 7 shown to lead to exon 7 skipping and to create a premature termination codon a few residues downstream in exon 8 (Arad et al., 2005; Sabourdy et al., 2009). Absence of the transmembrane domain of LAMP2 protein due to deletion of exon 9 possibly leads to loss of protein function. Reduction in LAMP2 protein is believed to disrupt the intracytoplasmic trafficking and lead to accumulation of autophagic material and often glycogen in skeletal and cardiac muscle cells by a mechanism that is not yet understood (D'souza et al., 2014).

Our patient's mutation was reported initially in two genetically unrelated families by Arad et al. (2005), one with a sporadic case of juvenile onset HCM and the other with the classic triad of Danon disease symptoms i.e. mental retardation, myoskeletal weakness and HCM. Bertini et al. (2005) described two unrelated male patients with the c.928G > A *LAMP2* mutation but with different clinical presentation: the first patient manifested hypotonia in infancy and a severe life-threatening hypertrophic obstructive cardiomyopathy, while the second patient presented with proximal limb weakness, mild mental retardation and HCM. The second's patient mother had received a heart transplant at the age of 40 after being diagnosed with HCM at the age of 33. Sabourdy et al. (2009), presented a male patient carrying the c.928G > A mutation, with muscle weakness, learning difficulties in early infancy, and mild cardiac involvement. Phenotypic heterogeneity of this mutation was also confirmed by Burusnukul and his colleagues (2008), who presented an unusual pediatric case of a 16 months old male child manifesting autism, motor delay and voltage hypetrophy of left and right ventricles (Table 1).

Our proband presented initially to the clinic with severe dilated cardiomyopathy and atrial fibrillation. Her CPK, AST and ALT levels were within normal range, but she had elevated LDH levels and enlarged liver. Her twin sister manifested also heart failure due to dilated cardiomyopathy. AST, ALT and LDH levels were elevated, liver was enlarged but the CK levels were normal. Neurological evaluation was normal for both sisters and according to their clinical picture, it was difficult to diagnose Danon disease (Table 1). The genetic test though, revealed the presence of the *LAMP2* mutation c.928G > A, in both patients, that has been strongly correlated with Danon disease as described above. Although the clinical manifestations of the disease in males are severe, the clinical presentation in female patients with Danon disease may be variable, ranging from asymptomatic to very severe (Fu et al., 2016). This variety of clinical manifestations in heterozygous women might originate from unfortunate X inactivation of the non mutated chromosome or from inhomogeneous distribution of LAMP2 loss, due to zonal inactivation of the X chromosomes (Bottillo et al., 2016; Fu et al., 2016; Maron et al., 2009; Miani et al., 2012). Random variable X inactivation may account for the differences in phenotypic severity between female members in the same family (Toib et al., 2010). Untested genetic influences like the presence of modifier genes may also affect the phenotypic expression of the *LAMP2* mutations contributing to the phenotypic variation of Danon disease.

Although dilated cardiomyopathy is a common phenotypic manifestation for females with Danon disease, in this study, it is the first time that DCM is associated with the *LAMP2* mutation c.928G > A, supporting the phenotypic heterogeneity observed in unrelated patients with the same *LAMP2* mutation and the different phenotypic manifestation between males and females. The prognosis associated with cardiomyopathy due to *LAMP2* mutations is poor. The onset of the disease during adolescence is followed by a rapid progression toward end-stage heart failure in adulthood, often resulting in death (Arad et al., 2005; Nishino et al., 2000). In the absence of a family history, it may be challenging to early diagnose the disorder in female patients and genetic testing is critical for early molecular identification of the disease and consideration for timely intervention with heart transplantation as treatment option.

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