Identification of Two Novel LAMP2 Gene Mutations in Danon Disease

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ABSTRACT

Background: Danon disease is a rare X-linked inherited disorder characterized by massive left ventricular hypertrophy, skeletal muscle dystrophy, and mental retardation. The disease is caused by mutations in the LAMP2 gene encoding for lysosome-associated membrane protein-2.

Methods: Two young male patients with hypertrophic cardiomyopathy, characterized by marked, concentric left ventricular hypertrophy, elevated levels of creatine kinase, and manifest limb-girdle muscular dystrophy in 1 case, were investigated. Genetic screening included direct sequencing of the whole coding sequence of the LAMP2 gene.

Results: Genetic analysis identified 2 novel LAMP2 gene mutations. In Family A, a G-A transition (c.962G > A) leading to a nonsense mutation at codon 321 (p.Trp321Ter), and in Family B, a one-nucleotide inser-

Danon disease (OMIM 300257), first described by Moris J. Danon in 1981, is a rare X-linked disorder characterized by cardiomyopathy, skeletal myopathy, and mental retardation. Although the skeletal myopathy is generally mild and the mental retardation is variable, it is the hypertrophic cardiomyopathy that predominates the clinical picture and determines the outcome. Cardiac symptoms in male carriers usually begin during adolescence, and patients generally die of heart failure in their third decade. Women are less severely affected than men; the onset of the disease is in late adulthood and shows a slower progression. Women have been reported to exhibit a dilated rather than a hypertrophic cardiomyopathy. Blood creatine kinase (CK) levels are usually elevated (ranging from 300 to 3000 U/L).

Danon disease is caused by a primary deficiency of lysosome-associated membrane protein-2 (LAMP-2), whose gene (LAMP2) maps to chromosome region Xq24. The LAMP-2 protein structurally consists of a small cytoplasmic tail with a lysosomal membrane targeting signal, a transmembrane domain, and a large intraluminal head with 2 internally homologous domains connected by a hinge region. LAMP-2 proteins coat the inner surface of the lysosomal membrane (mainly the LAMP-2B isoform) and are also...
tion (c.973insC) leading to a full frame-shift (p.Pro324→X) was detected in exon 8 of the LAMP2 gene. Family screening identified 8 mutation carriers, with 4 nonpenetrant cases and 3 additional, probably affected family members without DNA diagnosis. The cardiac phenotype was hypertrophic cardiomyopathy in all cases, including female mutation carriers. Five disease-related deaths occurred in the families, at an average age of 33±16 years, which was clearly lower in male than in female patients (28±7 vs 42±25 years). A high prevalence of arrhythmias or conduction abnormalities was also observed.

Conclusions: The reported 2 novel LAMP2 gene mutation carrier families, one of them being one of the largest reported to date, highlight the high rate of disease-related death at an early age and a high prevalence of arrhythmias or conduction abnormalities.

abundant on the plasma membrane (mainly the LAMP-2A and LAMP-2C isoforms). The LAMP2 open reading frame consists of 1233 nucleotides and encodes 410 amino acids. Exons 1-8 and part of exon 9 encode the luminal domain; the remainder of exon 9 encodes both the transmembrane domain and the cytoplasmic domain. Alternative splicing close to the 3′-end of the primary transcript generates 3 isoforms that differ in the transmembrane and cytoplasmic domains. Because of the rarity of the disease, awareness and clinical knowledge are scarce with regard to the phenotypic manifestation of the syndrome. In this work, we detail the clinical phenotype of 2 families with Danon disease due to 2 novel LAMP2 gene mutations that presumably caused the disease in the 2 families.

Patients and Methods

Case history of patients

Family A. In Family A, the index patient, a Rumanian boy (Fig. 1, subject III:1 in Family A), came to medical attention at the age of 12 years because of a heart murmur. His electrocardiogram (ECG) (Fig. 2A) showed a sinus rhythm with a short PR interval and a wide QRS complex with delta waves resembling the Wolff-Parkinson-White pattern. Echocardiography revealed massive asymmetric left ventricular (LV) hypertrophy, predominantly of the free wall (interventricular septum thickness: 30 mm, and LV free wall thickness: 39 mm), preserved LV ejection fraction (LVEF: 64%), systolic anterior movement of the mitral valve, severe obstruction of the LV outflow tract with a peak systolic gradient of 178 mm Hg, and severe mitral insufficiency (Fig. 2C, and Table 1). The hypertrophy involved the right ventricle, too.

At the age of 14 years, progressive muscle weakness developed. The clinical assessment showed an asthenic body constitution (body mass index: 16.14) and proximal atrophy of the scapulohumeral muscles. He had a moderate mental retardation with an IQ of 48 (Raven scale) and affective and cognitive immaturity. The neurologic findings revealed a proximal motor deficit, severe muscular atrophy with deltoid and triceps pseudohypertrophy, bilateral talus varus, and osteoendinous hyporeflexia. The laboratory findings indicated elevated levels of transaminases (serum glutamate-pyruvate transaminase [GPT]: 183 U/L [normal range: 2-41 U/L], serum glutamic oxaloacetic transaminase [GOT]: 376 U/L [normal range: 2-38 U/L]), creatine phosphokinase (CK): 1236 U/L (normal range: 24-270 U/L), and lactic dehydrogenase (LDH): 833 U/L (normal range: 40-300 U/L). On the basis of the above findings, limb-girdle muscular dystrophy was diagnosed.

At the age of 15 years, still with preserved LV function (LVEF: 62%), an implantable cardioverter defibrillator (ICD) was implanted as primary prophylaxis for sudden cardiac death (SCD) according to recent guidelines. Three years later, the patient presented several episodes of atrial flutter, with variable 3:1, 4:1, 6:1 atrioventricular (AV) block, responding to cardioversion. In the last year of his 11-year-long follow-up, the proband (now 23 years old) progressed into a dilated phase, exhibiting LV dilatation (LV end-diastolic diameter [LVEDD] raised from 30 to 41 mm) and a decrease of the LVEF from 64% to 35%. There is also echocardiographic evidence of thrombi in the left atrium and ventricle, and because of this oral anticoagulant treatment was started. The patient has chronic atrial fibrillation with a low ventricular response rate of 61 beats/min.

Family screening revealed a rich family history. The maternal grandmother (Fig. 1, subject I:2 in Family A) had a non-obstructive hypertrophic cardiomyopathy, heart failure of New York Heart Association functional class III, and chronic atrial fibrillation. A VVI pacemaker was implanted at the age of 44 years as antidysrhythmia protection. Death occurred at 60 years because of heart failure. The mother of the index patient (Fig. 1, subject II:1 in Family A) has ECG changes with negative T waves in V3-V6, but no clinical signs of Danon disease. The
In Family B, the index patient was a Hungarian boy (Fig. 1, subject II:1 in Family B), who was first admitted at the age of 14 years because of exercise-induced tachycardia. ECG showed a sinus rhythm, a short PR interval, and LV hypertrophy. Echocardiography revealed nonobstructive hypertrophic cardiomyopathy with an LV wall thickness of 16 mm and a normal LVEF (73%). The laboratory findings included elevated enzyme levels (CK: 729 U/L and LDH: 1149 U/L), with a normal CK-MB fraction (41 U/L, 5.6%) and elevated transaminases (GOT: 240 U/L and GPT: 190 U/L).

On follow-up, elevated CK levels persisted in the range 650-1200 U/L, with normal MB fraction and troponin levels, but signs or symptoms of muscle wasting or weakness did not develop. Follow-up echocardiography recorded an increase in LV wall thickness (LV wall diameter 26-28 mm). Audiology revealed a left-sided mild neural hearing loss; the ophthalmology was normal. Mild mental retardation was present.

At the age of 15 years, cardiac arrest occurred on mild exercise, due to ventricular fibrillation (the first recorded rhythm in the ambulance), which was successfully defibrillated. For secondary prevention, a DDD ICD was implanted. After ICD implantation, an inappropriate ICD discharge occurred because of supraventricular tachycardia. An electrophysiology (EP) study was performed that revealed a concealed septal-parahisian accessory pathway and an inducible orthodromic AV tachycardia under isuprel infusion. Non-sustained atrial tachycardia (AT) was also induced. Ablation of the accessory pathway was attempted, and proved successful, but a second ablation was necessary 3 years later, after multiple episodes of ICD inappropriate discharges induced by supraventricular tachycardia. A detailed EP study was again performed and a second accessory pathway was eliminated successfully at the anterior segment of the mitral ring.

At the age of 19 years, LV dilatation was noted (LVEDD: 46 mm and LV end-systolic diameter [LVESD]: 37 mm) with a mild decrease in EF (EF: 44%-48%), and the patient was evaluated for heart transplantation. On oxy-spiroergometry, the aerobic capacity was measured as 14.2 mL/kg/min. In a 6-minute walk test, he walked for 330 m without desaturation. An endomyocardial biopsy was also performed, which showed severe cardiomyocyte hypertrophy with extensive sarcoplasmatic vacuolization compatible with Danon disease (Fig. 3, A-D).
Four months later, at the age of 20 years, left-sided hemiparesis occurred because of an acute ischemic stroke, which showed spontaneous regression without thrombolysis. Soon after, biventricular heart failure and low-output syndrome developed, aggravated by bronchopneumonia, and the patient died because of intractable heart failure. The last echocardiography showed an LVEDD of 61 mm, an LVESD of 49 mm, and an EF of 40%.

The mother of the index patient (Fig. 1, subject I:1 in Family B) was first assessed at the age of 44 years when she exhibited normal echocardiography parameters (interventricular septum: 10 mm, LV free wall: 10 mm, LVEDD: 47 mm, LVESD: 31 mm, and EF: 63%). An ECG showed a sinus rhythm of 44/min with biphasic T waves in V2-V3.

After having 2 syncopal episodes at the age of 48 years, AT and a junctional escape rhythm were found, leading to the performance of an EP study. This study showed multiple ATs with divergent mechanisms and localizations. Altogether 3 different ATs were inducible, and 2 of these tachycardias were ablated successfully (one right atrial cavotricuspidal-isthmus-dependent flutter and another focal left AT with a posterolateral origin). The most likely mechanism of the third tachycardia was a macroreentry, propagating around the left atrial appendage, but this arrhythmia was not mappable completely because of spontaneous termination.

**Figure 2.** Electrocardiogram (ECG) and morphologic appearance of Danon disease. A 12-lead resting ECG of the index patient in Family A (subject III:1, Family A) (A) and his cousin (subject III:5, Family A) (B) showing a sinus rhythm, a short PR interval, and delta waves in leads I, aVL, and V4-V6, resembling Wolff-Parkinson-White syndrome. Paper speed 25 mm/s, calibration 5 mm/mV. (C) Parasternal short axis view of transthoracic echocardiography of the index patient of Family A (subject III:1, Family A), showing extreme concentric left ventricular (LV) hypertrophy, with an LV wall thickness of 27-36 mm, predominantly involving the left free wall. (D) Parasternal long axis view of transthoracic echocardiography of the cousin of the index patient (subject III:5, Family A), showing extreme concentric LV hypertrophy, with an LV wall thickness of 20-36 mm, predominantly involving the left free wall. (E, F) Cardiac magnetic resonance imaging of the cousin of the index patient (subject III:5, Family A), showing massive hypertrophy of the left ventricle (LV free wall: 42.3 mm, interventricular septum: 30 mm) with late gadolinium enhancement revealing focal changes in the basal and apical septum, and in the free wall of the left ventricle.
Table 1. Demographic and clinical characteristics of clinically or genetically affected family members of Family A and Family B

<table>
<thead>
<tr>
<th>Family</th>
<th>Subject</th>
<th>Sex</th>
<th>Age (y, at diagnosis and/or last FU or death)</th>
<th>Length of FU (y)</th>
<th>Died of disease</th>
<th>Clinically affected</th>
<th>Genetically affected</th>
<th>Cardiac phenotype</th>
<th>LVmax (mm)</th>
<th>Clinical course</th>
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<tr>
<td>A</td>
<td>I:2</td>
<td>Female</td>
<td>44/60</td>
<td>16</td>
<td>Yes</td>
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<td>Yes</td>
<td>HCM</td>
<td>15.4</td>
<td>AF, PM implantation, died of heart failure</td>
</tr>
<tr>
<td>A</td>
<td>I:1</td>
<td>Female</td>
<td>NA/44</td>
<td>NA</td>
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<td>No</td>
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<td>Negative T waves in V4-V6</td>
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<td>A</td>
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<td>Male</td>
<td>23/34</td>
<td>11</td>
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<td>HCM</td>
<td>25</td>
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<td>ND</td>
<td>HCM</td>
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<tr>
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<td>Male</td>
<td>12/23</td>
<td>11</td>
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<td>Yes</td>
<td>Yes</td>
<td>HCM</td>
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<td>Yes</td>
<td>Normal</td>
<td>10.7</td>
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<tr>
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<td>Male</td>
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<td>Muscle dystrophy, AF, ICD implantation, progression into dilated phase</td>
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<td>B</td>
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<td>Normal</td>
<td>10</td>
<td>CK rise, PSVT, accessory pathway, ICD implantation, died of HF at age 29</td>
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<tr>
<td>B</td>
<td>I:1</td>
<td>Male</td>
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<td>6</td>
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<td>Yes</td>
<td>Yes</td>
<td>HCM</td>
<td>28</td>
<td>CK rise, PSVT, accessory pathway, ICD implantation, died of intractable heart failure</td>
</tr>
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</table>

AF, atrial fibrillation; AVB, high-degree AV block; CK, creatine kinase; FU, follow-up; HCM, hypertrophic cardiomyopathy; ICD, implantable cardioverter defibrillator; LVmax, maximal left ventricular wall thickness; NA, not applicable; ND, not done; PM, pace maker; PSVT, paroxysmal supraventricular tachycardia; RF, radiofrequency; SCD, sudden cardiac death; WPW, Wolff-Parkinson-White.

Figure 3. Histology of LAMP2 vacuolar cardiomyopathy. (A) Hypertrophied cardiomyocytes with myofibre disarray and sarcoplasmic vacuolization. Haematoxylin-eosin; original magnification ×20. (B) Periodic acid-Schiff–positive (proved diastase-resistant) sarcoplasmic inclusions (arrowheads), irregular widespread sarcoplasmic vacuolization, and marked hypertrophy of the cardiomyocytes. The sarcoplasmic glycogen content does not seem to be increased. Periodic acid-Schiff, original magnification ×40. (C) Autophagic vacuoles among myofibrils, with disrupted limiting membranes. Electron microscopy, original magnification ×7500. (D) Perinuclearly located autophagic vacuoles (asterisks), with discontinuous limiting membranes. The vacuoles contain glycogen particles (arrowhead) and degenerated cellular membranes. The autolysosome filled with dense material (arrow) corresponds to the PAS-positive inclusions shown in (B). Bar represents 1 μm.
The younger brother of the index patient (Fig. 1, subject II:2 in Family B) had no symptoms at the age of 12 years, and exhibited normal ECG and echocardiography.

Genetic analysis

The family members and patients’ care givers gave informed consent to molecular genetic analyses. Genomic DNA was isolated from peripheral blood samples. All 9 exons and flanking intronic sequences of the LAMP2 gene were amplified by polymerase chain reaction with primers published in the literature. PCR products were directly cycle-sequenced using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Austin, TX) on an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA).

Bioinformatics

Nucleotide changes are reported according to the Ensembl database (release 76—August 2014) using LAMP2-001 (ENST00000200639) as a reference sequence.

Results

Two novel mutations in the LAMP2 gene were identified in the 2 index cases. In the index patient of Family A, a G-A transition was detected (c.962G > A) in exon 8 of the gene (Fig. 4A), which changes the tryptophan coding TGG codon to a stop codon TAG, at codon 321 (p.Trp321Ter, nonsense mutation). In the proband of Family B, a 1 bp insertion in position 973 (c.973insC) is revealed (upper sequence) as compared with the normal sequence (lower sequence).

Genetic screening of Family A and Family B

In Family A, DNA was available from 7 family members. With the exception of the nephew of the proband (subject III:6 in Family A), they all proved to be carriers of the mutation (Fig. 1). In Family B, DNA was available from the mother (subject I:1 in Family B) and the brother (subject II:2 in Family B). The proband’s mother carried the mutation, whereas the brother did not (Fig. 1).
Clinical course of LAMP2 gene mutation carrier family members

Altogether, 13 family members were screened in the 2 families, whereas genetic analysis was possible in 10 family members. Eight family members proved to be carriers of either LAMP2 gene mutation. Of the 8 mutation carrier family members, 4 proved to be clinically not affected (in terms of the development of cardiomyopathy at the last follow-up). In addition to the 4 penetrant cases with DNA diagnosis, we identified 3 additional family members with a suggestive manifestation of Danon disease (subjects II:3, II:6, and III:6 in Family A). This gave a total of 7 patients in the 2 families with proven or likely diagnosis of the disease.

The average age at the onset of the disease was 21 ± 11 years, which was clearly lower in male than in female patients (16 ± 5 vs 31 ± 18 years; no statistical comparison was made because of the small sample sizes). The cardiac manifestation was hypertrophic cardiomyopathy in all cases, including female patients. Atrial fibrillation was observed in 5 cases, and multiple ATs were observed in 1 nonpenetrant case. Sustained or nonsustained supraventricular tachycardia was noted in almost all cases. Pacemaker implantation was necessary in 3 cases due to a high-degree AV block. An ICD was implanted in 3 cases, for primary, and 1 for secondary prevention.

Of the 7 clinically manifest patients, 5 patients (71%) died at an average age of 33 ± 16 years. The age at death was clearly lower in male than in female patients (28 ± 7 vs 42 ± 25 years). The average time span from the time of diagnosis to death was 8 ± 5 years. The mode of death was heart failure in 4 cases and SCD in 1 case. An additional aborted SCD occurred in another case.

Discussion

We report here the identification of 2 novel LAMP2 gene mutations in 2 families with Danon disease. To the best of our knowledge, one of these families, Family A, is one of the largest families with Danon disease reported to date, in terms of the number of affected family members with a proven DNA diagnosis. The characteristics of a small number of large families and a slightly larger number of small families have been published. However, in the majority of large families, a significant number of the family members were already deceased at the time of the report, with no material available for DNA analysis and therefore obviating the possibility of establishing a definitive DNA diagnosis.

In these cases, the suggestive clinical status cannot be proven, which leaves uncertainty about the clinical phenotype described. In the 2 families, we identified altogether 8 gene mutation carriers (6 in Family A and 2 in Family B) that clearly helped us to draw conclusions about the clinical course of the disease in the families.

In both index cases, the clinical manifestation of the disease was typical for Danon disease, with extreme concentric LV hypertrophy, pre-excitation on the ECG, muscle dystrophy or CK rise, and variable mental retardation. The cardiac phenotype in the affected family members, including female family members, was hypertrophic cardiomyopathy, and a high prevalence of arrhythmias or bradyarrhythmias, necessitating pacemaker implantations. Five disease-related deaths occurred in the families at a young age.

Although radiofrequency ablation procedures have been used to treat arrhythmias in Danon disease, results of an electrophysiological study are surprisingly rarely reported in such patients. In one of our patients (subject II:1, Family B), an EP study was performed twice, with unusual findings. In 1 EP study, the accessory pathway was mapped to the anterior region of the left AV ring, which is a very unusual location of accessory pathways, present in 0%-1% of patients with Wolff-Parkinson-White syndrome. In addition to the induction of the AV tachycardia, nonsustained atrial arrhythmias were also inducible. Further to this, another genetically affected family member (subject I:1, Family B), without manifest cardiomyopathy, also underwent an EP study because of multiple ATs with macroreentrant circuits and focal origin involving both atria. This observation raises the possibility of the presence of a disease substrate affecting the atria, and pointing to atrial arrhythmias as one of the early manifestations of the disease.

The characteristics of the 2 novel mutations, that we have identified, are in agreement with literature data. The professional version of the Human Gene Mutation Database (www.hgmd.org) lists 68 LAMP2 gene mutations causing Danon disease or glycogen storage disease 2h. Approximately one-quarter of the reported LAMP2 mutations are point mutations, the majority (approximately 80%) of them being nonsense mutations. Another one-third of the mutations are small insertions and/or deletions, leading to a frame-shift. An additional one-quarter of the mutations represent splice site mutations, with a variable and unpredictable expression. Gross deletions and insertions are also reported in the literature. Accordingly, the overwhelming majority of the mutations reported to date represent loss of function mutations that lead to a complete or almost complete loss of LAMP-2 protein expression. One of the mutations we identified, p.Trp321Ter, is a nonsense point mutation, presumably leading to a premature stop codon at codon 321. The other mutation that we described, p.Pro324fs+24X, is a one-base pair insertion that is predicted to lead to a frame-shift and incorporation of 24 amino acids before activation of a hidden stop codon. Neither mutation has been described previously.

In conclusion, we have described 2 families with novel LAMP2 gene mutations causing Danon disease. Both mutations were predicted to lead to a truncated LAMP-2 protein that presumably lacks the transmembrane and cytoplasmic domains. We observed a markedly malignant phenotype in both families, characterized by a large proportion of disease-related death.

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Disclosures

The authors have no conflicts of interest to disclose.

References


