LAMP2 Microdeletions in Patients with Danon Disease

Running Title: Yang et al; LAMP2 microdeletion in Danon disease

Zhao Yang, MD, PhD 1, Birgit H. Funke, PhD, FACMG 2, Linda H. Cripe, MD 3, G. Wesley Vick III, MD, PhD 1, Debora Mancini-Dinardo, PhD 2, Liana S. Peña, MS 1, Ronald J. Kanter, MD 4, Brenda Wong, MD 3, Brandy H. Westerfield, MS 1, Jaquelin J Varela, B.Se 1, Yuxin Fan, MD, PhD, FACMG 1, Jeffrey A. Towbin, MD 3, Matteo Vatta, PhD 1,5

1Department of Pediatrics (Cardiology) and John Welsh Cardiovascular Diagnostic Laboratory, Baylor College of Medicine, Texas Children’s Hospital, Houston, TX; 2Laboratory for Molecular Medicine Partners Center for Personalized Genetic Medicine 65 Landsdowne St, Cambridge, MA; 4Heart Institute, Department of Pediatrics and Pediatric Cardiology, Cincinnati Children’s Hospital Medical Center, Cincinnati, Ohio; 5Department of Pediatrics, Duke University Medical Center, Durham, North Carolina; 5 Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX

Correspondence to:
Matteo Vatta, PhD
Pediatrics (Cardiology)
Baylor College of Medicine/Texas Children’s Hospital
1102 Bates St., F.C. 430.04
Houston, Texas 77030
Phone: 832-824-4153
Fax: 832-825-4153
E-mail: mvatta@bcm.edu

Abstract

Background: Danon disease is an X-linked dominant disorder characterized by the clinical triad of hypertrophic cardiomyopathy (HCM), skeletal myopathy and variable mental retardation. Pathologically, autophagic vacuoles are noted in both skeletal and cardiac muscle. It exhibits an X-linked dominant mode of inheritance and males are severely affected, while females develop milder and later-onset cardiac symptoms. Danon disease has been associated with mutations in the LAMP2 gene located at Xq24, typically resulting in splicing defects or protein truncation affecting the lysosome-associated membrane glycoprotein 2 (LAMP2). Because of its rarity, the full spectrum of genetic mutation resulting in Danon disease has not been elucidated.

Methods and Results: We analyzed three males with clinical and pathological findings consistent with Danon disease. Comprehensive mutational analysis failed to yield detectable products for selected LAMP2 exons and genomic DNA deletion was suspected. Genomic junction fragment PCR analysis in Case-1 identified a novel Alu-mediated 34kb microdeletion encompassing the entire 5’UTR and exon-1 of LAMP2. In Case-2 and -3, junctional PCR and Southern Blot analyses mapped the breakpoint to a MIRb and (TA)n simple repeats present in intron-3, which determined a 64kb and a 58Kb deletion, respectively, thereby ablating exons-4-10. Western blot analysis confirmed the absence of LAMP2 in protein extract from lymphocytes of index Case-2.

Conclusion: This is the first report of Danon disease caused by microdeletions at Xq24, which functionally ablate LAMP2. The microdeletion mechanism appears to involve one Alu-mediated unequal recombination and two chromosomal breakage points involving TA-rich repeat sequences.

Key words: LAMP2, HCM, Danon, Alu, (TA)n simple repeat, MIRb, MER21B, LIMA4A, Xq24, lysosome, vacuoles
Introduction

Hypertrophic cardiomyopathy (HCM) is a complex cardiac disease with unique pathophysiological characteristics and a wide spectrum of morphologic, functional and clinical features. In these patients, the interventricular septum and left ventricular posterior wall are thickened (usually asymmetric septal hypertrophy) and hypercontractile systolic function is noted along with diastolic dysfunction. Systolic anterior motion of the mitral valve and left ventricular outflow tract obstruction may also occur. Although HCM has been considered a relatively uncommon cardiac disease, the prevalence of echocardiographically defined HCM in a large cohort of apparently healthy young adults selected from a community-based general population was reported to be 1 in 500 persons. While sporadic cases are common, familial disease (FHC) with autosomal dominant inheritance predominates.

Most of the genes involved in HCM encode proteins of the sarcomere, which is a complex structure with an exact stoichiometry and multiple sites of protein-protein interactions. The encoded proteins include myofilament proteins, β-MyHC (MYH7), which was the first gene to be identified, α-MyHC (MYH6), ventricular myosin essential light chain 1 (MYL3) and ventricular myosin regulatory light chain 2 (MYL2); thin filament proteins, cardiac actin (ACTC1), cardiac Troponin T (TNNT2), cardiac Troponin I (TNNI3), cardiac Troponin C (TNNC1), and α-Tropomyosin (TPM1); a myosin-binding protein, cMyBP-C (MYBPC3), and titin (TTN). These mutations have been described in detail in a number of reviews. Recently, mutations in the caveolar protein caveolin-3 (CAV3), as well as in several sarcomeric Z-line genes such as Cysteine and glycine-rich protein (CSRP), Telethonin (TCAP), myozenin-2 (MYOZ2), myopalladin (MYPN),
vinculin (*VCL*), alpha-actinin-2 (*ACTN2*) and LIM domain-binding protein 3 (*LDB3*) were identified in patients with HCM. In addition, mutations in junctophilin 2 (*JPH2*) and phospholamban (*PLN*), which modulate calcium ion release from the sarcoplasmic reticulum and calcium influx from the plasma membrane L-type calcium channels, have been reported to cause HCM.

Further adding to the spectrum of HCM-causing genes in human subjects, mitochondrial and metabolic abnormalities have been reported. In 2004, Van Driest *et al.* screened 389 unrelated HCM patients for mutations in the 7 sarcomeric genes and identified potential mutations in less than 40%. Mutations in certain genes encoding metabolic / lysosomal storage proteins have been identified in a small proportion of patients, including the gene encoding the γ2 subunit of AMP-activated protein kinase (*PRKAG2*), and the alpha-galactosidase (*GLA*) gene associated with Fabry disease. In addition to Fabry disease, another lysosomal storage disorder, known as Danon disease, is associated with the development of skeletal myopathy, variable mental retardation with intracytoplasmic vacuoles containing autophagic material and glycogen in skeletal and cardiac muscle cells, and presents with HCM. Mutations in the *LAMP2* gene, which encodes the lysosome-associated protein-2 and maps to Xq24, were initially identified as the cause of Danon disease. Subsequently, Danon disease was classified in the subgroup of autophagic vacuolar myopathies (AVMs), once it was recognized that sarcolemmal proteins and basal lamina are associated with the vacuolar membranes. Affected males usually present with HCM at puberty or even earlier, while most female carriers develop dilated cardiomyopathy (DCM) rather than HCM during adulthood (as late as their 40s). Skeletal muscle biopsy usually reveals numerous glycogen containing
Mental retardation, although usually mild and of variable degree, has been noted in some patients.\textsuperscript{10,14-19} Female carriers also have skeletal myopathy and mental retardation less commonly than affected males.\textsuperscript{14-19}

To date, the great majority of reported mutations in the \textit{LAMP2} gene represent loss of function mutations (small insertions and/or deletions leading to frameshift and nonsense mutations).\textsuperscript{12-27} These are predicted to result in complete absence of the protein through nonsense-mediated decay (NMD), in which transcripts containing premature termination codons are targeted. This causes rapid degradation, protecting the organism from deleterious dominant-negative or gain-of-function effects of resulting C-terminal truncated proteins.\textsuperscript{28} Large genomic deletions are generally suspected in genes in which small loss of function mutations are common; however, they are frequently missed due to short range PCR-based mutation detection technologies, particularly for autosomal genes. Here, we report three individuals with Danon disease who carry large genomic deletions involving the \textit{LAMP2} gene. We present the first evidence of chromosomal rearrangements affecting the \textit{LAMP2} genomic sequence \textit{via} a homologous unequal recombination, an increasingly recognized mechanism in cardiac genetic diseases.\textsuperscript{29}

\textbf{Materials and Methods}

\textbf{Patient evaluation}

All patients were evaluated by physical examination, chest radiography, electrocardiography (ECG), echocardiography, and magnetic resonance imaging (MRI). Left ventricular size and function were evaluated by M-mode and two-dimensional
Doppler and color Doppler echocardiographic images, and cardiac arrhythmias were studied by 24-hour Holter monitoring. Serum creatine kinase levels were measured to evaluate the association of skeletal myopathy.

**Mutational analysis**

After informed consent, blood was obtained for lymphoblastoid cell line immortalization and DNA extraction, as regulated by the Baylor College of Medicine Institutional Review Board (IRB). Genomic DNA was amplified by PCR (Invitrogen, Carlsbad, CA) using primers designed to amplify the coding exons of the *LAMP2* gene as well as the upstream and downstream genomic sequences encompassing the *LAMP2* gene (primer sequences available on request) and purified the PCR products using exonuclease I (USB, Cleveland, OH) and shrimp alkaline phosphatase (Roche, Indianapolis, IN). DNA sequence analysis was performed using Big Dye terminator chemistry (v3.1) and an ABI 3730 genetic analyzer (Applied Biosystems, Foster City, CA) as previously described.

**Junction fragment PCR and sequencing**

Sequential PCR using primers upstream and downstream of the *LAMP2* gene was performed and potential breakpoints were mapped. Long fragment PCR product was obtained using primers flanking the deleted region. Amplified PCR product was purified and directly sequenced as described above. Reference genomic DNA sequence is NM_013995 (NCBI) and ENSG00000005893 (www.ensembl.org).

**Southern blot analysis**
Briefly, 15 μg genomic DNA was digested with SpeI endonuclease overnight at 37°C. The digested DNA was resolved on a 0.7% agarose gel and transferred to a 0.45 μm nylon membrane (Pall Corporation, Pensacola, FL) with 0.4N NaOH solution following standard Southern blot transfer procedure. 25ng of purified probe (flanking Lamp2 exon 2 or 6) DNA (1μl) was then labeled with 5μl of 50μci 32P-dCTP using 4μl High Prime solution (Roche Applied Sciences, Mannheim, Germany), purified with QiaQuick Nucleotide Removal Kit (Qiagen, Valencia, CA) and used to probe the Southern blot.

Western blot analysis

Lymphoblastoid cell lines were homogenized in lysis buffer containing 1% Triton X-100. The lysates were clarified by centrifugation at 20,000g for 30 minutes and the Protein concentration was determined by BCA protein assay kit (Pierce, Rockford, IL) and equal amounts of protein were subjected to SDS-PAGE (Invitrogen) followed by transfer to nitrocellulose membranes by electrotransfer, as previously described. Proteins were detected as previously described. Monoclonal antibodies against LAMP2 (Antibody # H4B4) and LAMP-1 (H4A3) were obtained from the Developmental Studies Hybridoma Bank, University of Iowa.

Results

Clinical Characterization

Clinical presentation of Case-1 (0014): A 12-year old Caucasian male presented with recurrent rest and exertional syncopal episodes beginning at 4 years of age. He was in special classes for reading and math due to “visual and auditory processing problems”,

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but otherwise was without other health issues including hypertension. His family history was without any cardiac or skeletal muscle disease or sudden death. Echocardiography demonstrated moderate concentric left ventricular hypertrophy, without left ventricular outflow tract obstruction (Figure 1A). His ECG demonstrated sinus bradycardia, a short PR interval (100-110 ms) with possible pre-excitation, an interventricular conduction delay (QRSd=110-118 ms), marked left lateral lead ST segment depression, and suggestive of severe LVH (Figure 1B). Electrophysiological testing demonstrated a fasciculoventricular fiber as manifested by constant short HV interval during atrial extrastimulus testing (Figure 1C). Electron microscopy performed on right ventricular apicoseptal endomyocardial biopsy demonstrated myeloid bodies and punctate PAS-positive inclusions (Figure 1D), while ultrastructural analysis demonstrated mildly to moderately dense glycogen vacuoles, perinuclear myeloid bodies, and lamellated lysosomes (Figure 1E), consistent with the diagnosis of Danon disease.

Clinical presentation of Case-2 (00732): This 12 year old Caucasian male presented at first with gastrointestinal (GI) problems. Skeletal muscle weakness was noted on exam and laboratory evaluation revealed a high CPK. Muscle biopsy was performed and it showed dystrophic features that along with the clinical findings of a mild proximal weakness and an elevated CK could suggest the diagnosis of limb-girdle muscular dystrophy despite the presence of basophilic cytoplasmic granular inclusions. Of note, pre-excitation was observed on the pre-operative electrocardiogram. The family history was unremarkable for any individuals with known neuromuscular disorders. Two years later he underwent Holter monitoring and stress test, which identified premature
ventricular contractions (PVC) with variable coupling intervals before, during and after exercise, while ventricular preexcitation persisted throughout the test (Figure 2A).

At 17 years of age, the subject was referred for ophthalmologic evaluation, after suspecting a diagnosis of Danon disease, and he presented with a peripheral pigmented retinopathy, consistent with the diagnosis of Danon disease, despite no decline in visual function. The patient presented with mild learning delays needing special educational classes and vocational training instead of college level functioning. On examination a progressive skeletal myopathy was appreciated. Echocardiogram demonstrated concentric left ventricular hypertrophy. The patient’s diagnosis was changed to Danon disease after LAMP-2 gene mutational analysis. Because the electrocardiogram continued to show evidence of ventricular preexcitation, consistent with Wolff-Parkinson-White (WPW) syndrome, he underwent radiofrequency ablation of 3 out of the 5 mapped accessory atrio-ventricular connections.

At 21 years of age, routine MRI using a 3.0 Tesla magnet and an 8-channel phased-array cardiac coil confirmed persistent concentric left ventricular hypertrophy without obstruction. There was depressed systolic dysfunction (EF = 37%; Z score = -5.9). Gadolinium delayed enhancement examination demonstrated severe LV myocardial free wall and inferoseptal nonperfusion and fibrosis (Figure 2B). The MRI findings were similar to those described by Piotrowska-Kownaka et al., in a case of Danon disease.32 Echocardiography confirmed the severe concentric left ventricular hypertrophy with no left ventricular outflow tract obstruction, mild to moderately depressed biventricular systolic function, and trivial to mild mitral regurgitation (Figure 2C). Routine ECG evaluation identified sinus rhythm and ventricular preexcitation with short PR interval.
(96ms) and wide QRS complexes (194ms), premature ventricular complexes, and prolonged QT interval (QTc = 499ms). Holter monitoring showed frequent PACs and PVCs, and a short run of tachycardia (24 beats) (Figure 2D). Two months later, while shopping he experienced a generalized seizure and was unresponsive, followed by flaccid right side paresis. In the emergency room, he had runs of ventricular tachycardia (VT) lasting three hours. Neuroimaging demonstrated the presence of an extensive CNS infarction. Patient was declared brain dead and support was withdrawn.

**Clinical presentation of Case-3 (0013):** This 25-year old Caucasian male with no evidence of skeletal muscle weakness but with developmental delay/learning disabilities underwent full clinical evaluation and the echocardiogram demonstrated left ventricular hypertrophy, while muscle biopsy demonstrated myopathy, glycogenosis, and intracytoplasmatic vacuoles. Baseline ECG analysis demonstrated third-degree AV-block, and the patient underwent implantation of a pacemaker. Metabolic studies revealed elevated serum levels of CPK, ALT and AST.

**Molecular Characterization**

**Molecular analysis of Case-1 (0014):** The DNA was sent to our laboratory to confirm the result of a previously performed *LAMP2* gene mutational test, which had identified a possible genetic deletion of exon-1. We confirmed that the PCR analysis failed to amplify exon-1 of the *LAMP2* gene. Primers were designed to perform long-fragment PCR (available upon request), followed by sequence analysis, which located the breakpoints ~25.5kb upstream of the start codon (within the 5'UTR) and ~8.9kb
downstream of start codon (within intron 1) of the \textit{LAMP2} transcript (ENST00000371345) of the \textit{LAMP2} gene (\textbf{Figure 3A}). This \~34.4kb deleted region includes the first coding exon of the \textit{LAMP2} gene and \~9kb of the first intron; no other known genes are present in this deleted region.

Repeatmasker analysis of the \textit{LAMP2} genomic sequence encompassing the aforementioned breakpoints identified \textit{Alu}-repeat elements of distinct subfamilies in both 5’- and intron-1 regions of \textit{LAMP2} (\textbf{Figure 3A}). The breakpoint sequence identified a 45-bp core motif with 100% homology to an \textit{Alu}Sx-repeat at the 5’-UTR and one \textit{Alu}Sx1-repeat element in intron-1 of \textit{LAMP2} (\textbf{Figure 3B}). Overall, the two \textit{Alu}-repeat elements shared 88% identity.

\textbf{Molecular Analysis of Case-2 (00732):} Comprehensive open reading frame/splice site \textit{LAMP2} gene mutational analysis followed by direct DNA sequencing was performed on the patient genomic DNA. However, we were unable to amplify exons-4 through 10 of the \textit{LAMP2} gene. Therefore, a significant genomic DNA deletion was suspected and therefore sequential PCR primers were designed to identify the possible chromosomal deletion. Using long-fragment PCR, the breakpoints were localized \~14kb and \~72kb downstream of the transcript LAMP2-201 (ENST00000371345) of the \textit{LAMP2} gene, deleting intron-3, exons-4-10 and part of the 3’UTR (\textbf{Figure 3A and 3C}). Computational analysis of the \textit{LAMP2} genomic sequence identified a \((\text{TA})_n\)-simple repeat element at the 5’-breakpoint and a MER21B repeat element highly rich in TA (62%) at the 3’-breakpoint in our index case-2. Southern blot analysis using the available family members confirmed the deletion, which was present only in index case-2, consistent with
a *de novo* mutation (*Figure 3D*). We found that, as expected, a probe against *LAMP2* exon 2 detected a ~3kb band in the patient sample as opposed to a 7kb band in normal family samples (*Figure 3D*). Furthermore, a probe against *LAMP2* exon 6 detected no band in the patient sample as opposed to a ~14kb band in normal family sample controls (*Figure 3D*). To determine whether this deletion affects the translation of the *LAMP2* gene, we also performed western blot analysis for LAMP2 and LAMP1 (another lysosomal membrane protein) proteins, using GAPDH as a loading control. LAMP2 protein was undetectable in the index Case-2 sample, although its expression was similar among all other family members (*Figure 3E*). In contrast, there was no difference in the expression of LAMP1 protein in the patient and his family members (*Figure 3E*). We also performed microsatellite analysis and confirmed that the proband and his relatives are from the same family (data not shown). This supports the concept that this is a *de novo* deletion in the *LAMP2* gene of this patient which is not present in other family members.

**Molecular analysis of Case-3 (0013):** The DNA sample for Case-3 was sent to our laboratory to confirm the previously performed *LAMP2* gene mutational test, which had identified a possible genetic deletion of exons-4-10 of *LAMP2*. No parental DNA samples were available for analysis. We confirmed that the PCR analysis failed to amplify exons-4-10 of the *LAMP2* gene and, similar to Case-2, we located the breakpoints at ~14kb downstream of the start codon (within intron-3) and ~78kb downstream of start codon (within 3'-UTR) of the *LAMP2* gene (*Figure 3A*). The deleted region contained only the *LAMP2* gene and no other known genes were involved.
Computational analysis of the \textit{LAMP2} genomic sequence encompassing the breakpoints identified in index Case-3 revealed a MIRb repeat element at the 5’-breakpoint and the LIMA4A repeat element at the 3’-breakpoint families of \textit{LAMP2}, both rich in TA (68.5%). The Southern blot analysis was performed using the DNA from the index Case-3 and we compared it with a control individual from the family of Case-2. The analysis detected differentially-sized bands in the patient compared to control sample using a probe against \textit{LAMP2} exon 2 (Figure 3F). With a probe against \textit{LAMP2} exon 6, we were able to detect a band in the control individual but not in our Case-3 subject (Figure 3F).

\textbf{Discussion}

Danon disease is an X-linked disorder that affects multiple organs and should be strongly considered in individuals with HCM and skeletal myopathy. The \textit{LAMP2} gene, located on chromosome Xq24, is the only gene known to cause Danon disease, and the vast majority of the identified missense, nonsense, small insertions/deletions result in impaired mRNA splicing.\textsuperscript{12-27} In particular, even missense mutations such as the V310I, which has been identified in multiple patients, was found to affect RNA processing, resulting in a frameshift.\textsuperscript{15,27} Although \textit{LAMP2} has been cloned from humans for more than two decades,\textsuperscript{33} the role the gene plays in human disease is still uncertain. The need for a functionally normal LAMP2 in the cell was also recently suggested by studies employing the monoclonal antibody H4B4, which could block human LAMP2 and induce apoptosis in human microvascular endothelium \textit{in vitro}.\textsuperscript{34}

Here, to our knowledge, we present the first evidence of \textit{LAMP2} microdeletions involving repetitive sequence motifs such as \textit{Alu}-mediated or TA-rich repeat elements...
and predicting the ablation of a functional LAMP2 product. These chromosomal rearrangements caused the deletion of critical portions of the gene, thus preventing it from yielding a functional protein. In particular, the Alu-mediated microdeletion in Case-1 caused the ablation of the entire 5’UTR regulatory sequence, the first exon, and a large part of the first intron. This predicts the absence of a functional transcript as it occurs in mutations causing altered splicing that affect the first exon/intron boundaries. 21 The microdeletions in Case-2 and -3, mediated by TA-rich repetitive sequences, both ablate exons-4-10, although their breakpoints differ slightly from each other.

The (TA)n and MER21B elements at the breakpoint involved in the deletion in Case-2 shared 45.4% identity, while the MIRb and LIMA4A elements, present at the breakpoint in Case-3 microdeletion shared 46.3% identity. Because of the low identity rate, the exact mechanism leading to the microdeletions in Case-2 and -3 remains elusive. Breakpoints occurring within TA-rich segments have been previously reported to encompass complex chromosomal rearrangements due to inexact or illegitimate homologous recombination mechanisms. 35,36 However, elements rich in TA have been also involved in fragile site-based genomic rearrangements. 37 At this stage, we do not have sufficient elements to dissect the detailed nature of the mechanism leading to LAMP2 microdeletions in Case-2 and -3.

Nonetheless, the absence of exons-4-10 causes the lack of any canonical stop codon and predicts the deficiency of functional transcripts, as predicted by NMD, although cryptic termination sites could have occurred. Therefore, to test this hypothesis we also performed western blot analysis, which confirmed that the microdeletion caused a functional knockout in the affected subject, but did not influence LAMP1 expression.
Although all microdeletions identified in this report affect different regions of the LAMP2 gene, they probably originate from a similar homologous unequal recombination mechanism. However, the sequence identity of the repeated elements causing microdeletions in LAMP2, appears to be low compared to the canonical repeat sequence-mediated genomic rearrangements, typically sharing >90% identity. This perhaps explains why LAMP2 large deletions have been encountered less commonly than small mutations. Nonetheless, homologous unequal recombination is a well described mechanism on the X chromosome, as we recently demonstrated in a Barth syndrome subject presenting with an Alu-mediated mechanism totally ablating TAZ and functionally ablating DNL1L.29 In addition, our findings confirm that the Xq24 region is prone to chromosomal rearrangements as recently demonstrated in premature ovarian failure.38 Genomic deletions are often observed in genes for which small loss-of-function mutations are common. These deletions can be difficult to detect as re-sequencing is usually performed using amplicons that are smaller than the deleted interval. Heterozygous deletions on autosomes are therefore “masked” by the presence of the wild-type allele, which raises the question whether some forms of dilated and hypertrophic cardiomyopathy may also stem from the deletion of one of the two autosomal alleles that are generally required to exert a normal function, leading to a copy number variation in these subjects. Because the LAMP2 gene is located in the X-chromosome, hemizygous mutations and large deletions can be detected through common techniques such as PCR in males.

In conclusion, this is the first report describing three LAMP2 microdeletions in patients with Danon disease, cardiac hypertrophy and cardiac rhythm disturbance. These findings
broaden the range of genomic alterations observed in Danon disease, focusing attention on the increasing complexity of genetic testing for these subjects.

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**Conflict of Interest Disclosures:** None

**Reference:**


Figure Legends

Figure 1: Cardiac evaluation in subject 0014. (A) Echocardiographic parasternal short-axis (right panel) and long-axis views (left panel) of the index case demonstrates moderate concentric left ventricular hypertrophy without left ventricular outflow tract obstruction. (B) Electrocardiogram of the proband at 12 years of age showing sinus bradycardia, short PR interval (100-110 ms), interventricular conduction delay (QRSd=110-118 ms), massive LVH, marked left lateral lead ST segment depressions, and possible preexcitation in a nonspecific pattern. (C) Intracardiac electrograms which identified a fasciculoventricular fiber, as manifested by constant short HV interval. (D) Electron microscopy performed right ventricular apicoseptal endomyocardial biopsy demonstrated rare slightly hypertrophic cardiomyocytes and punctate PAS-positive inclusions in multiple cells that were generally perinuclear and were diastase resistant. These were not associated with identifiable vacuoles. (E) Ultrastructural evaluation showed glycogen present within the cytoplasm and also in some vacuoles. The vacuoles were of mild to moderate density and few in number. Multiple myocytes exhibited perinuclear myeloid bodies - lamellated lysosomes- that indicate myocyte injury and degeneration.

Figure 2: Cardiac evaluation in subject 00732. (A) Electrocardiogram of the proband demonstrating ventricular preexcitation, consistent with WPW syndrome. (B) Steady-state free precession MRI short axis image (right panel) shows severe concentric left ventricular hypertrophy. Gadolinium-delayed contrast MRI four-chamber image (left panel) indicates presence of severe left ventricular free wall and inferior septal fibrosis.
(arrowheads) (C) Echocardiographic parasternal short-axis (right panel) and long-axis views (left panel) of the index case demonstrates severe concentric left ventricular hypertrophy without left ventricular outflow tract obstruction. The echocardiogram also demonstrates depressed biventricular systolic function and mild mitral regurgitation. (C) ECG demonstrating ventricular preexcitation, short PR interval, wide QRS, premature complexes, and prolonged QT interval, consistent with WPW.

Figure 3: Molecular characterization of Danon disease subjects. (A) Detailed description of the *LAMP2* locus showing the coding exons, the alternative stop codons, the SpeI restriction map, and the repetitive sequences involved in the three microdeletions present in index cases 0014, 00732 and 0013. Number of base pairs indicates their location relative to the *LAMP2* start codon. (B) Sequence analysis of the junctional fragment PCR product identifying the breakpoint ~25kb upstream and ~9kb downstream of the start codon, detected in subject 0014. (C) Sequence analysis of the junctional fragment PCR product identifying the breakpoint ~14kb and ~72kb downstream of the start codon, and characterizing the ~58kb microdeletion, which ablates two SpeI restriction sites in index case 00732. (D) Southern blot analysis of the index case and the available family members performed using the E6 (top panel) and E2 (bottom panel) probes recognizing exon-2 and exon-6 of the *LAMP2* gene as described in the panel with the expected fragment sizes. Samples were loaded according to the order in the pedigree shown on top. As expected, the E2 probe detected a 3kb band in the proband and a 7kb band in the other relatives, while probe E6 detected no band in subject 00732 and identified a 14kb band in the other family members. (E) Western blot analysis
of LAMP2 or LAMP1 proteins. GAPDH was used as loading control. LAMP2 protein was undetectable in the patient sample, while it was normally expressed in the other relatives. However, no difference was identified in the expression of LAMP1 in patient 00732 and his family members. (F) Southern blot analysis of patient 0013 using probe against exon-2 (E2) or against exon-6 (E6) of the LAMP2 gene. The E2 probe identified two differently-sized bands, while the E6 probes detected the expected band in control, but not in the sample from the index case.
Fasciculoventricular fiber
Figure 1

Myeloid bodies

Vacuolated glycogen
Figure 3

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