

Original Article

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LAMP2 variants in four Chinese children with Danon disease: clinical and molecular analysis in a monocentric cohort

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Abstract

Background: Danon disease is an X-linked disorder caused by variants in the lysosome-associated membrane protein-2 (*LAMP2*) gene located on Xq24. Due to its inheritance in an X-chromosome dominant manner, males typically experience more severe manifestations than females. **Method:** The whole exome sequencing was conducted on a cohort of 218 children diagnosed with hypertrophic cardiomyopathy; four children with hypertrophic cardiomyopathy carrying the *LAMP2* variants were diagnosed at our centre. Variants in the *LAMP2* gene were summarised, and their pathogenicity and conservation were analysed using bioinformatics methods. A retrospective analysis of genotype-phenotype associations was also conducted in conjunction with previously reported cases. **Results:** Four patients with Danon disease were diagnosed in our single centre by gene sequencing; they all presented with myocardial hypertrophy as the initial manifestation. Both male patients manifested symptoms from infancy, while disease onset in the two female cases occurred below the average age reported for females. Through gene sequencing, a total of four variants were identified in these four patients, including one splicing variant: c.865-1G>C, one loss of heterozygosity variant: loss1 exon:4-9), one frameshift variant: c.973delG(p.(L325Wfs×21)), and one stop codon variant: c.467T>G(p.(L156*)). **Conclusion:** This study identified four patients with *LAMP2* gene variants, thereby enriching the documented genetic landscape of *LAMP2*-associated disorders. Bioinformatics analyses corroborated the pathogenicity of these variants. Additionally, we emphasised that women with suspected Danon disease should be thoroughly evaluated, and the possibility of implantable cardioverter defibrillator implantation and heart transplantation should be considered and discussed as early as possible.

Introduction

Hypertrophic cardiomyopathy is now increasingly recognised as having a complex genetic aetiology. Hypertrophic cardiomyopathy exhibits a diverse range of clinical presentations and stands as the most prevalent cause of sudden cardiac death among young individuals. The estimated prevalence in the general population ranges from approximately 1:200 to 1:500.¹ Despite current progress, the precise aetiology of hypertrophic cardiomyopathy remains enigmatic; nevertheless, genetic factors play a pivotal role in its pathogenesis. The annual risk of sudden cardiac death in hypertrophic cardiomyopathy varies from 0.5% to 2%.² Echocardiography and cardiac MRI synergistically contribute to diagnosing index cases and conducting family screenings, whereas genetic testing enables the identification of affected individuals lacking the characteristic hypertrophic cardiomyopathy phenotype. Although pharmacological therapy serves as the cornerstone for alleviating hypertrophic cardiomyopathy symptoms, its efficacy in averting cardiac diseases and associated complications specific to hypertrophic cardiomyopathy remains limited. On rare occasions, patients with advanced pathology may present treatment-refractory symptoms and compensatory imbalances, necessitating the implantation of a left ventricular assist device or heart transplant.³ Danon disease (OMIM *300257), a rare X-linked dominant disorder associated with pathogenic variants in the lysosomal associated membrane protein-2 (*LAMP2*) gene,⁴⁻⁶ is one of the metabolic causes of hypertrophic cardiomyopathy. About 1% of hypertrophic cardiomyopathy patients carry variants in the *LAMP2* gene.⁷⁻⁹ Besides, patients with Danon disease also manifest non-cardiac symptoms, including skeletal myopathy, intellectual disability, psychiatric disorder, hepatic involvement, and retinopathy.¹⁰

The *LAMP2* gene (OMIM#309060) encodes three isoforms, including *LAMP-2A*, *LAMP-2B*, and *LAMP-2C*, which have common N-terminal domains but different C-terminus. Variants linked to Danon disease are mainly located in the N-terminal region of *LAMP2*.^{5,11,12} The three *LAMP2* isoforms, especially *LAMP-2B*, play a key role in lysosomal autophagy. *LAMP2* is mainly involved in mediating the fusion of autophagosomes, with lysosomes to form

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autolysosomes, facilitating lysosomal protein degradation. *LAMP2* deficiency causes impaired autolysosome formation and lysosomal protein degradation, which leads to accumulation of glycogen granules and autophagic vacuoles in cardiomyocytes and skeletal muscle cells.^{13,14}

Due to haploinsufficiency, male patients usually exhibit an early onset in infancy or childhood, characterised by severe cardiac and non-cardiac phenotypes, while female patients manifest a later onset with a slower progression of isolated hypertrophic cardiomyopathy in general.^{4,15} Danon disease is difficult to identify and distinguish from other causes of hypertrophic cardiomyopathy in female patients. In a study involving 60 young female patients with end-stage cardiomyopathy, seven cases of Danon disease (12%) were identified.¹⁶ Female patients usually progress into end-stage heart failure between ages 20–40 years, unless they undergo heart transplantation.¹⁰ Early diagnosis of Danon disease in female patients is challenging but crucial for improving survival rate and promoting genetic counselling.

In this study, four patients with Danon disease were diagnosed in 218 children with hypertrophic cardiomyopathy, including two males and two females. The two male paediatric patients were previously reported in our research centre¹⁷ and we now provide an update on their prognosis. Notably, two female patients exhibited a unique atypical phenotype. Through gene sequencing, we identified four different variants in the *LAMP2* gene, including two de novo variants. These variants expand the mutational spectrum of *LAMP2* and provide new insight into the disease mechanism underlying Danon disease.

Materials and methods

Subjects

Molecular diagnostic testing at our centre identified 218 children with hypertrophic cardiomyopathy. Prior to genetic testing, all study participants or their legal guardians provided written informed consent. The study was approved by the Ethics Committee of the Children's Hospital of Nanjing Medical University. Clinical trial number: not applicable.

Peripheral blood collection and deoxyribonucleic acid library preparation

Two millilitres of peripheral venous blood samples were collected from the patients and their parents and siblings. Genomic deoxyribonucleic acid was isolated using the Qiagen DNA Mini Kit. Subsequently, 1–3 µg of genomic deoxyribonucleic acid was fragmented to an average size of 150 base pairs using an S220 Focused-ultrasonicator instrument (Covaris, USA).

Whole exome sequencing

The study was approved by the institutional review board of the Affiliated Children's Hospital of Nanjing Medical University. The proband and their family members' genome deoxyribonucleic acid were extracted from their peripheral blood and quantified with the Nanodrop 2000 (Life Technology, USA). Whole exome sequencing was performed on an Illumina HiSeq 2000 (Bio-Rad, Hercules, CA, USA) using 2 x 100 base pairs paired-end reads. Variants with allele frequencies higher than 1% were filtered out. The whole exome sequencing data were processed in accordance with the best practice of The Genome Analysis Toolkit. The resulting variants were annotated using ANNOTATE VARIATION software. The

minor allele frequency was annotated using databases Genome Aggregation Database, Single Nucleotide Polymorphism Database, 1000 Genomes minor allele frequency (Chinese), Exome Aggregation Consortium, Human Gene Mutation Database, Clinical Variation Database, and an in-house minor allele frequency database. Variants were also interpreted and classified using the American College of Medical Genetics and Genomics/Association for Molecular Pathology standards and guidelines and the Clinical Variation Database standards and guidelines. The candidate variants were validated by Sanger sequencing, and the diagnostic variants were defined as "pathogenic" or "likely pathogenic" according to the American College of Medical Genetics and Genomics guidelines and included the variants of uncertain significance. The functional significance of unpublished variants was evaluated by Sorting Intolerant From Tolerant, Polymorphism Phenotyping version 2, and Mutation Taster. The original contributions presented in the study are publicly available.

Sequence analysis of *LAMP2*

We extracted genomic deoxyribonucleic acid from blood. We amplified each exon and flanking intronic regions with the following primers (the number in the name of the primer indicates exon; F represents forward and R reverse; and sequences are written in 5' to 3' orientation): E4F, ATACTTTACTCACCATTGTGCTC; E4R, CAACCTTTGGAAAAGAATGATGTTGT; E6F, GC TTCAGTTATTAACATCAACCCCAA; E6R, GACAAAGTCTAG ATACTTAATGGTGCT; E9F, TCCAGCATGATGGTGCTTGAG AC; E9R, TGACGACAACCTTCCTTGTCGCC; IVS6F, CTCACACT GCTCTACT; IVS6R, ATGCTGATGTTCACTT; E8F, CAATCTCA GCTACTGGGATG; E8R, ATCTGAAATGCTCCAGAC; E4F, GGC CATCAGAATCCATT; E4R, CTTGTACAAGAACATCCCA. We directly sequenced the amplified fragments with both forward and reverse primers using BigDye Terminator Cycle Sequencing Kit (PE Biosystems), and then electrophoresed the samples using an ABI PRISM 310 Genetic Analyzer (PE Biosystems). We used PAC clone DJ318C15 sequence (accession number AC002476) as a reference, which includes the entire genomic sequence of *LAMP2* gene in complementary orientation.

Quantitative polymerase chain reaction confirmation of the copy number variation

The primer pair sequences are shown in Supplementary Table S1. Samples for quantitative polymerase chain reaction were assayed in triplicate using the Takara SYBR Green with glyceraldehyde-3-phosphate dehydrogenase genomic content used as an endogenous control for normalisation of the data. The $\Delta\Delta C_t$ comparison method was used to measure relative deoxyribonucleic acid content on the QuantStudio™ 3 instrument (ThermoFisher).

Bioinformatics analysis

The bioinformatics analyses focused on protein-altering variants (missense, nonsense, frameshift, and splice-site). Variants were annotated with the following public and internal bioinformatics/genetics resources. Sequencing results were analysed using DNASTAR (Madison) software. MGI sequencing adapters were screened with cutadapt and low-quality reads (<80-base-pair), while single-nucleotide polymorphism and insertion-deletion variants were detected by the parameter driver of Sention software. Single-nucleotide polymorphism variants were then analysed using ANNOTATE VARIATION software for further

annotation of variants (<http://annovar.openbioinformatics.org/en/latest/>). The frequency of the variant in the population was evaluated by comparison with variants from the following databases: dbSNP (<https://www.ncbi.nlm.nih.gov/snp/>), Genome Aggregation Database (<https://gnomad.broadinstitute.org/>), and Iranome (<http://www.iranome.ir/>).

Results

Clinical characteristics

We documented four paediatric patients with hypertrophic cardiomyopathy harbouring heterozygous *LAMP2* gene variants, comprising two male and two female cases. Their median age at onset was three years and eight months, with the youngest being three months and the oldest fourteen years. The mean age of onset was 10.5 years in female children and 3.5 months in male children, indicating that females developed the condition later than males. All four children had hypertrophic cardiomyopathy as their earliest clinical symptom, with skeletal muscle involvement in patient 3 and patient 4. All four youngsters had anomalies in electrocardiographic conduction and increased aminotransferases, but they did not have any intellectual or retinal issues (Figure 2A, 2B, 3C).

Patient 1 presented with an onset age of 14 years. Laboratory investigations revealed marked elevations in cardiac biomarkers: high-sensitivity cardiac troponin I (144.97 pg/mL) and B-type

natriuretic peptide (86 pg/mL). Echocardiographic evaluation demonstrated concentric left ventricular hypertrophy characterised by significant thickening of both the interventricular septum and left ventricular posterior wall, with preserved left ventricular ejection fraction (74%). Following 24 months of combination therapy with metoprolol and coenzyme Q10, echocardiographic monitoring demonstrated maintained left ventricular ejection fraction (70–75%). Neurological evaluations revealed preserved cognitive function and muscle strength throughout the observation period. Concurrently, serial serum creatine kinase measurements consistently remained within normal physiological limits (50–310 U/L). Patient 2 exhibited earlier disease onset at age 7 years, with initial biochemical testing demonstrating significantly elevated B-type natriuretic peptide (1180 pg/mL). Echocardiographic evaluation revealed mild left ventricle/interventricular septum thickening accompanied by ventricular wall dyssynchrony, with measured left ventricular ejection fraction at 59%. After one week of combined treatment with vitamin B, metoprolol and coenzyme Q10, the high-sensitivity cardiac troponin I of patient 2 declined to 39 pg/mL, the B-type natriuretic peptide reduced to 425.1 pg/mL, and the left ventricular ejection fraction was improved (64.3%) (Table 1). The left ventricular ejection fraction was stable at 60–75% and the serum creatine kinase level was maintained at 50–310 U/L during the follow-up period, and no intellectual disability or myopathy changes were observed. As previously reported,¹⁷ patient 3 and patient 4 exhibited early-onset presentations with distinct clinical trajectories. Patient 3 presented at 4 months of age

Table 1. Clinical features, electrocardiograph, and echocardiogram findings of five patients

	P1	P2	P3	P4
Gender	Female	Female	Male	Male
Age at diagnosis	14y	7y	4m	3m
Family history	+	-	+	-
Death	-	-	+	-
HCM	+	+	+	+
IVS(mm)	-	7.4	15.4	44.5
LVPW(mm)	-	7.2	15	28.6
LVOT blood flow rate(m/s)	1.54	-	3.46	4.05
E/A ratio	>1	1.7	0.68	0.7
LVEF(%)	74	64.3	-	-
LVFS(%)	43	34.1	-	46
ECG abnormality	Premature ventricular contractions, ventricular preexcitation	ST-segment depression, T-wave inversion, shortened PR interval	Left ventricular hypervoltage, widespread ST-T changes	Left ventricular hypervoltage complete right bundle branch block
CK levels(U/L)	123	68	310	354
hsTnI(pg/ml)	144.97	39	83	-
BNP(pg/ml)	86	425.1	-	-
Inverted T wave	+	+	-	+
mental retardatio	-	-	-	-
skeletal muscle weakness	-	-	+	+

Abbreviations: ECG = electrocardiograph; IVS = interventricular; LVEF = left ventricular ejection fraction; LVPW = left ventricular posterior wall; HCM = hypertrophic cardiomyopathy; LVOT = Left ventricular outflow tract.

Table 2. Variations of *LAMP2* gene identified in this study

Patient	Nucleotide Change	Amino acid Change	Variation	Location	Zygosity	Variation Frequency	Co-segregation	ACMG	Reported
P1	c.865-1G>C	–	Splicing	IVS6	Het	<0.0005	unknow	P(PVS1+PS4+PM2)	PMID: 21415759
P2	loss1 (EXON: 4-9)	–	Loss of heterozygosity	E4-9	Het	<0.0005	De novo	LP(PVS1+PM2)	No
P3	c.973delG	p.L325Wfs*21	Frameshift	E8	Hemi	–	unknow	P(PVS1+PS1+PM2);	PMID: 25900304
P4	c.467T>G	p.L156*	Stop codon	E4	Hemi	–	De novo	P(PVS1+PS1+PM2+PS2)	PMID: 16144992

Abbreviations: P = Pathogenic; LP = Likely pathogenic; ACMG = American College of Medical Genetics and Genomics.

with acute respiratory distress (manifesting as cough and dyspnoea requiring urgent referral). Neurological assessment revealed reduced muscle strength. Cardiac enzymes showed an elevated serum troponin T level of 0.083 ng/ml, echocardiography showed left ventricular hypertrophy, and left ventricular outflow tract obstruction. Patient 4 was diagnosed with hypertrophic cardiomyopathy at 3 months during routine screening, and neurological examination showed decreased muscle strength. Echocardiography shows thickening of the ventricular septum and posterior wall of the left ventricle, and obstruction of the left ventricular outflow tract.

All four children were treated similarly with coenzyme Q10 for myocardial nutrition and long-term oral metoprolol for improvement of ventricular remodelling. Patient 3 was followed up for one year and two months when he developed bilateral lower extremity muscle weakness, which progressed gradually, and he died of heart failure at the age of two years and three months. The rest of the children are still being followed up, and so far, patient 4 has been coming to the hospital intermittently for treatment of recurrent respiratory infections (Table 1).

Molecular genetics analysis

A total of four variants were identified in these four patients, including one splicing variant: c.865-1G>C, one loss of heterozygosity variant: loss1 (exon:4-9), one frameshift variant: c.973delG(p.(L325Wfsx21)), and one stop codon variant: c.467T>G(p.(L156*)) (Table 2). According to silico (<https://nmdpredictions.shinyapps.io/shiny/>), all the nonsense and frameshift variants were fully degraded by the nonsense-mediated messenger ribonucleic acid decay pathway (Figure 1E).

Specifically, patient 1 was a heterozygote with variant of c.865-1(intron6)G>C, resulting in a frameshift variant. Exon 7 and neighbouring structures of the *LAMP2* gene and arrows show the location of splice site c.865-1G>C (Figure 1D). This alteration triggers the nonsense-mediated messenger ribonucleic acid decay pathway, leading to complete degradation of the messenger ribonucleic acid at the transcriptional level (Figure 1E). Patient 1's variant in the *LAMP2* gene was maternally inherited, and it is worth noting that her mother, maternal aunt, and maternal grandmother all died of 'heart disease' (the specific gene variant was not confirmed). There is a significant fragment deletion in exons 4–9 of the variant in patient 2, indicating an obvious pathogenicity (Figure 1C). Deletion of the *LAMP2* gene was suspected in patient 2 because of failure of polymerase chain reaction amplification. Copy number variant analysis of the whole exome sequencing data identified an interstitial deletion

encompassing the *LAMP2* gene (chrX:119209864-119582983) in the proband. We designed several primers in the *LAMP2* gene to confirm the deletion. Validation of this deletion by quantitative polymerase chain reaction confirmed heterozygous in the proband. Neither of patient 2's parents carries this variant, which is de novo. In patient 3, a notable finding was observed in the *LAMP2* gene, specifically a guanine deletion at position 973 (c.973delG) in exon 8. This particular variant led to the diagnosis of Danon disease in the patient. The variant in the *LAMP2* gene of patient 3 was confirmed to be of maternal origin and verified by Sanger sequencing. The deletion was predicted to induce a frame shift in the amino acid sequence, resulting in an altered protein product with a premature termination codon at residue p.345 (p.L325Wfsx21), thereby terminating translation prematurely. Another heterozygous variant c.77G > A (p. Ala26Val) in exon 2 of myosin heavy chain 7 was inherited from his phenotypically normal father. A hemizygous c.467T > G (p.L156*) in exon 4 of *LAMP2* was identified in patient 4, causing a premature stop codon, thereby confirming a diagnosis of Danon disease. This variant was not detected in his biological parents, indicating that it is likely de novo. The heterozygous c.5704G > C (p. Glu1902Gln) in exon 4 of myosin heavy chain 7 found in the patient was inherited from his asymptomatic mother. However, the two myosin heavy chain 7 variants identified are not the main cause of disease.¹⁷ The original contributions presented in the study are publicly available. These data can be found in the Leiden Open Variation Database: <https://databases.lovd.nl/shared/individuals?create,ID00454846,ID00454847,ID00454848,ID00454849>.

Discussion

To date, reports of the Danon disease phenotype have focused on severe outcomes in male patients, while fewer reports have been made of the phenotype in female patients, who appear to be less affected. In male patients, the cardiac manifestations are predominantly hypertrophic cardiomyopathy, whereas female patients have a comparable incidence of hypertrophic cardiomyopathy and dilated cardiomyopathy.²⁰ Specifically, males first experience symptoms at 12.1 years of age, require a heart transplant at 17.9 years of age, and die of the disease at an average of 19 years of age. In contrast, female patients had their first symptoms relatively late, at 28.1 years of age, received a heart transplant at 33.7 years of age, and died of the disease at an average of 34.6 years of age.¹⁰

Our cohort exhibited notable early disease manifestations, with a mean age at symptom onset of 10.5 years in females and 3.5

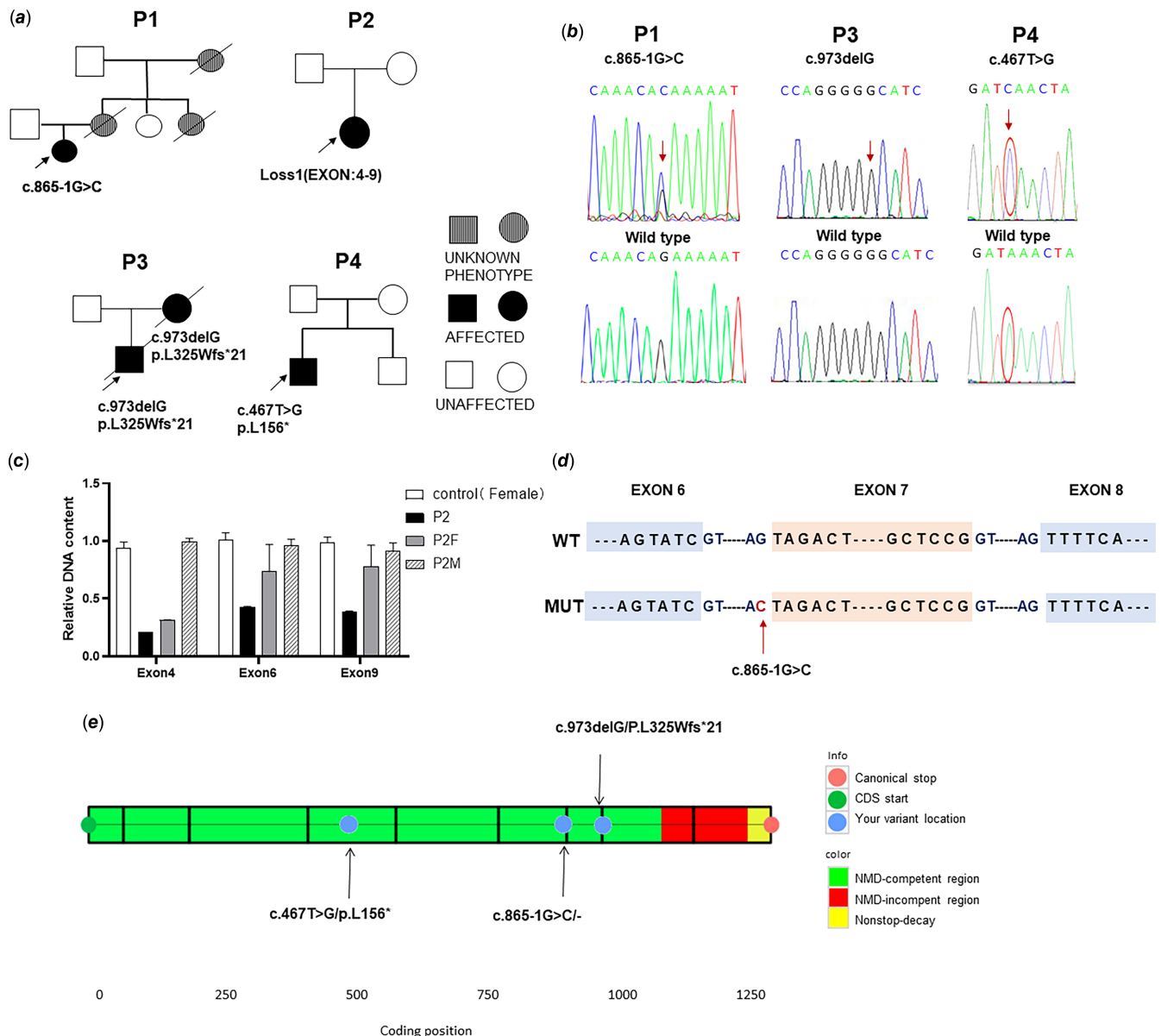


Figure 1. (Abbreviations: NMD, nonsense-mediated mRNA decay). **(a)** Familial analysis of the families of four patients. Arrows point out proband. Circles correspond to females. Squares correspond to males. **(b)** P1 was compound heterozygous with the c.865-1 (intron 6) G > C variant; genetic testing of P3 revealed a guanine deletion at position 973 (c.973delG) in exon 8 of the *LAMP2* gene; and a hemizygous c.467T > G (p.L156*) variant in exon 4 of *LAMP2* was identified in the P4, the figure placed is for the complementary strand (A > C). **(c)** *LAMP2* gene qPCR analysis of P2 revealed a heterozygous loss of exons 4-9 in *LAMP2*. **(d)** Exon 7 and neighbouring structures of the *LAMP2* gene. Arrows show the location of splice site c.865-1G>C. **(e)** All four variants triggered the NMD pathway.

months in males. This contrasts with reports from European and North American cohorts, where the mean age of initial symptom presentation was 12.1 years for males and 27.9 years for females.¹⁰ A Chinese cohort study involving 29 Danon disease patients reported comparable diagnostic ages of 7.2 ± 5.9 years for males and 9.4 ± 5.0 years for females.²¹ The substantially earlier age of symptom onset observed in our cohort compared to these reports suggests potential ethnically influenced modifiers of *LAMP2*-related phenotypic expression.

All four reported patients exhibited significant left ventricular hypertrophy at initial diagnosis. Notably, two male patients experienced severe disease progression: patient 3 ultimately succumbed to heart failure, while patient 4 has progressed to end-stage cardiomyopathy and currently awaits cardiac

transplantation. Comparative analysis with European cohorts reveals distinct phenotypic patterns. Females in European populations predominantly present with hypertrophic cardiomyopathy (70.3%) or dilated cardiomyopathy (29.3%), whereas males demonstrate higher hypertrophic cardiomyopathy prevalence (96.2%), with 43.8% progressing to end-stage cardiomyopathy.¹⁵ This study further corroborates previous reports of skeletal myopathy as a gender-specific manifestation, with literature indicating that $\geq 50\%$ of affected males develop skeletal muscle weakness.¹⁰ Consistent with these findings, both male patients (patient 3 and patient 4) in our cohort demonstrated concurrent skeletal muscle involvement alongside their primary cardiac manifestations. Notably, our Chinese paediatric cohort presented with hypertrophic cardiomyopathy as the initial clinical

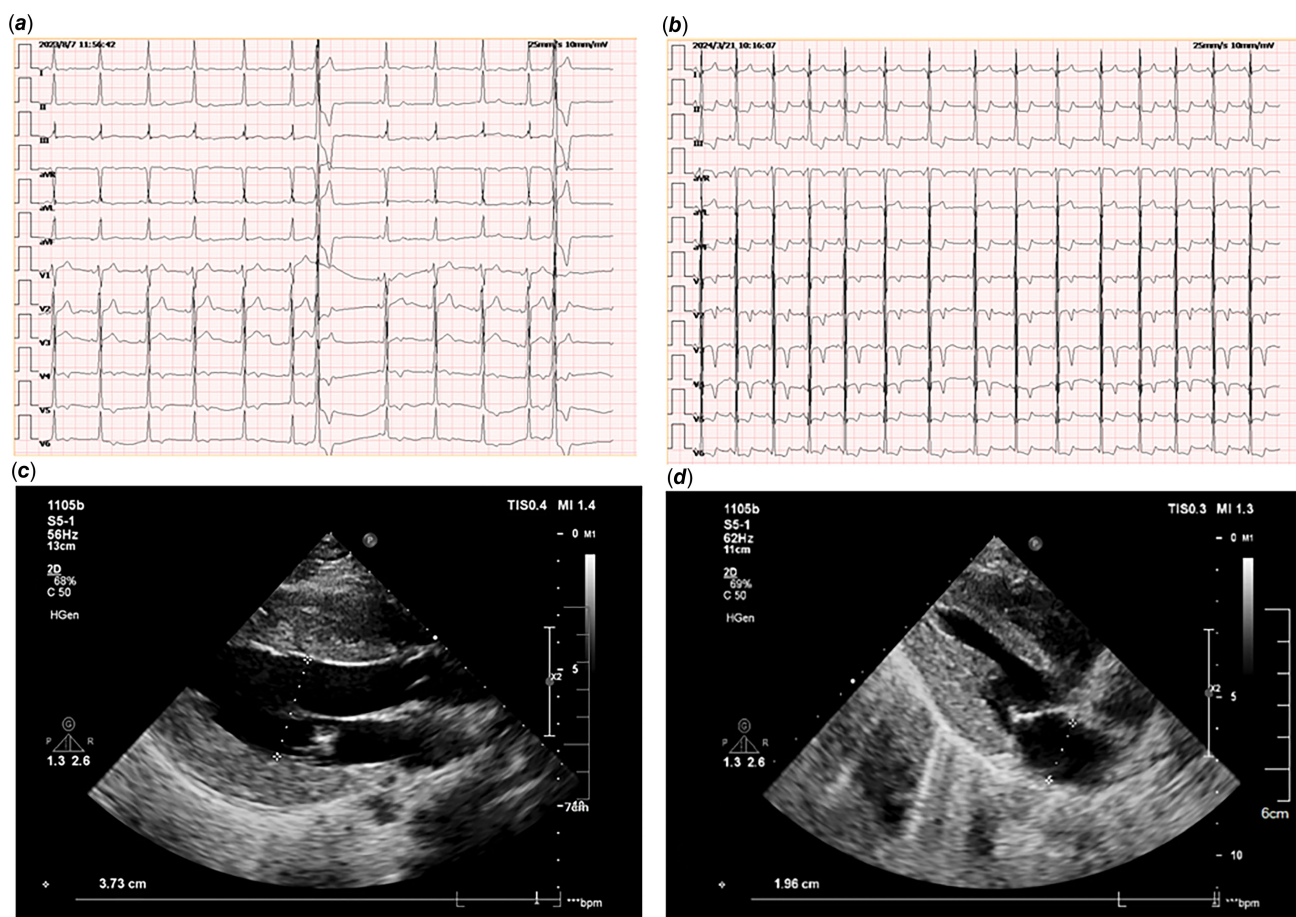


Figure 2. (Abbreviations: LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening). (a) The electrocardiogram of P1 at the time of his first hospitalisation (14 years of age) showed ventricular pre-systole and T-wave changes in some leads. (b) At the initial visit (7 years old), the electrocardiogram of P2 showed incomplete right bundle branch block and left ventricular hypertrophy. (c) P1 was admitted to the hospital (14 years of age) with cardiac ultrasound showing marked thickening of the interventricular septum and posterior wall of the left ventricle with a slight “fusiform” shape, LVEF 74%, LVFS 43%, and normal current cardiac function. (d) Cardiac ultrasound of P2 on admission (7 years of age) showed slight thickening of the left ventricle and interventricular septum, slight enhancement of myocardial echoes, poor structural definition, poor coordination of ventricular wall motion, and a small radius of the whole heart, and LVEF.

manifestation, contrasting with European data. This observation suggests potential ethnic differences in disease presentation, possibly indicating earlier onset or greater frequency of structural cardiac abnormalities in Chinese populations compared to European counterparts. Furthermore, the accelerated disease progression observed in male patients warrants particular clinical attention. However, these preliminary findings require validation through larger multicenter studies with extended follow-up periods.

Significantly, we diagnosed two male patients with younger onset and more severe clinical symptoms: patient 3 had his first episode at 3 months of age and died at 2 years and 3 months of age; patient 4 had his first episode at 4 months of age and is currently awaiting a heart transplant. Given the rapid progression of the disease, heart transplantation should be considered for both male and female patients as soon as they develop symptoms of heart failure.²² It was previously thought that male patients with mutations in the *LAMP2* gene were more severely affected than female patients due to the X-linked haploinsufficiency of the *LAMP2* gene.¹¹ However, using clinical data from two female Danon disease patients diagnosed at our single centre and querying previously reported cases, we found that female patients manifested myocardial hypertrophy at a significantly younger

age than anticipated, coupled with more aggressive disease progression relative to established clinical profiles. This reveals that female patients with suspected Danon disease should be thoroughly evaluated. Miani et al. report four cases of cardiac arrest in female patients carrying variants in the *LAMP2* gene, highlighting that this female population has a severe arrhythmic phenotype and a high risk of sudden cardiac death.²³ Three cases of Danon disease were reported: one woman received a heart transplant at the age of 27 years, a girl died at the age of 14 years while waiting for a heart transplant, and a third woman presented with typical advanced symptoms of Danon disease with severe cardiomyopathy and also required a heart transplant.²⁴ In a follow-up of two female patients at our centre, patient 1 has not yet shown significant progression, but her young age of onset and the fact that both her mother and grandmother died of heart disease may suggest a poorer prognosis. Malignant ventricular arrhythmias and cardiac insufficiency are usually the main cause of shortened life expectancy in patients and are characterised by a rapid progression to end-stage heart failure, and we therefore advocate careful assessment of cardiomyopathy in female Danon disease patients and in asymptomatic female relatives of male patients who are closely monitored for the development of potentially life-threatening early symptoms.

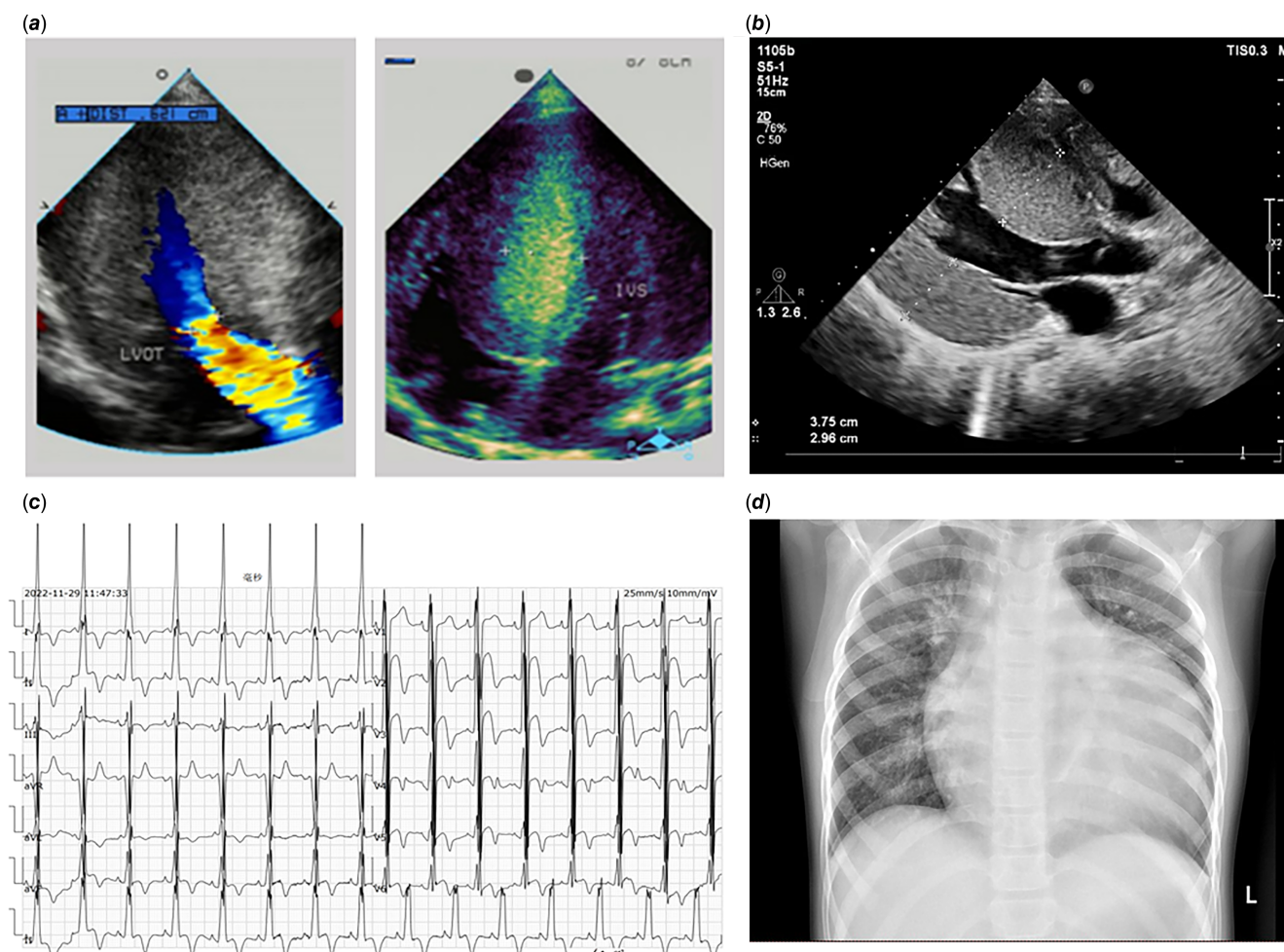


Figure 3. (Abbreviations: RVOT, right ventricular outflow tract; IVS, interventricular septum; LVEF, left ventricular ejection fraction). (a) Cardiac ultrasound of P3 (4 months of age) revealed an RVOT flow velocity of 3.46 m/s and a differential pressure of 53 mm Hg, left ventricular outlet obstruction, IVS hypertrophy (IVS = 15.4 mm). (b) The recent echocardiogram of P4 (11 years of age) showed significant left ventricular and septal hypertrophy with impaired diastolic function (E/A: 0.7) and normal systolic function (LVEF: 82.5%). (c) Recent electrocardiogram of P4 (11 years of age) showed left ventricular hypervoltage. (d) Chest X-ray of P4 (11 years of age) showed a markedly enlarged cardiac silhouette.

Both female patients (patient 1 and patient 2) currently exhibit isolated cardiac manifestations without detectable skeletal muscle involvement. The identified large intragenic deletion (exons 4–9) in *LAMP2* identified in patient 2 is predicted to disrupt critical functional domains, potentially resulting in complete ablation of the luminal region essential for protein stability. Notably, patient 2 developed cardiomyopathy at 7 years of age, significantly earlier than the reported mean onset age in female carriers, suggesting accelerated disease progression associated with extensive structural deletions.

In contrast, both male patients (patient 3 and patient 4) demonstrated severe multisystem phenotypes characterised by early-onset cardiomyopathy, markedly elevated creatine kinase levels, and histologically confirmed skeletal myopathy. Their nonsense variants (patient 3: c.973delG p.L325Wfs×21; patient 4: c.467T>G, p.L156*) introduce premature termination codons predicted to undergo nonsense-mediated messenger ribonucleic acid decay (Figure 1E), consistent with undetectable *LAMP2* protein expression on immunoblot analysis. This molecular mechanism correlates with the rapidly progressive clinical course observed in patient 3, who succumbed to cardiac failure at 2 years. The prolonged survival of patient 4 despite sharing similar

truncating variants may reflect residual function from partial preservation of the luminal domain or tissue-specific variations in nonsense-mediated messenger ribonucleic acid decay efficiency. These phenotypic discrepancies highlight the complex interplay between variant position and clinical outcomes in lysosomal storage disorders.

To date, more than 200 disease-causing variants have been reported in the *LAMP2* gene, including exons 1 through 9. Through gene sequencing, we identified one unreported de novo *LAMP2* variant. There are significant differences in disease prognosis and symptoms among Danon disease patients,¹⁵ but the corresponding genotype-phenotype correlation mechanisms remain unclear. This enlightens us that studying the molecular function of *LAMP2* isozymes to elucidate the mechanism of the *LAMP2* gene in Danon disease should be the focus of future work.

The diagnosis of Danon disease is difficult due to multiple non-cardiac symptoms and different clinical presentations. Currently, the main diagnostic criterion is genetic testing. However, it has been proposed that leukocyte immunoblotting analysis be considered as a screening method, given the prevalence of *LAMP2* protein abnormalities in different tissues in the vast majority of carriers of the mutant gene.²⁵ The case reported by

Hideaki Suzuki et al. showed high intrinsic T1 values and normal extracellular volume scores in two patients with Danon disease. This unique pattern of T1 mapping may reflect increased autophagic vacuoles within the cytoplasm.²⁶ Genetic analysis, combined with a non-invasive differential diagnosis, helps clinicians to distinguish Danon disease from other cardiac pathologies.

Patients with Danon disease have a rapid progression of disease and a poor prognosis, with available data showing that the natural life expectancy of the majority of patients with Danon disease rarely exceeds 30 years.²⁷ Although there is no specific gene therapy for Danon disease, adenovirus-mediated functional *LAMP-2B* transgene therapy has been achieved in a mouse model and its efficacy is being tested in adolescent male Danon disease patients.²⁸ And advances in the field of heart transplantation and defibrillators have helped to improve the overall prognosis of Danon disease. However, the exact mechanisms underlying the different phenotypes observed in this disease require further comprehensive studies. By analysing and sharing the clinical data and genetic information of four Danon disease patients from our single centre, we hope to increase cardiologists' awareness of this rare disease and raise awareness of the poor prognosis of female Danon disease patients.

Conclusion

We identified one new *LAMP2* variant associated with Danon disease, and our study broadens the genetic spectrum of Danon variants, providing four new cases for studying the relationship between genotype and phenotype. Compared with data from other ethnic populations, the age of symptom onset in our cohort was significantly earlier. In conclusion, Danon disease is phenotypically more prevalent in males, but the reported age of onset of cardiac hypertrophy in females is lower and the clinical symptoms are more severe than we would expect. This enlightens us that female patients with suspected Danon disease should be thoroughly evaluated and the possibility of implantable cardioverter defibrillator implantation and heart transplantation should be considered and discussed as early as possible.

Supplementary material. The supplementary material for this article can be found at <https://doi.org/10.1017/S1047951125101546>

Data availability statement. The original contributions presented in the study are publicly available. These data can be found in the Leiden Open Variation Database: <https://databases.lovd.nl/shared/individuals?create,ID00454846,ID00454847,ID00454848,ID00454849>.

Author contributions. JL conceived the study and was involved in designing the protocol, analysing the data, and interpreting the findings. YYQ, MC, CLW, JY, and SWY contributed to the design, analysis, and interpretation of the findings, and reviewed progressive drafts of the manuscript and proofread the manuscript. All authors read and approved the final version of the manuscript.

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Competing interests. No financial or non-financial benefits have been received or will be received from any party related directly or indirectly to the subject of this article.

Ethical standard. This study was approved by the institutional ethical committee of the Children's Hospital of Nanjing Medical University. Written informed consent was obtained from the parent.

Clinical trial number. Not applicable.

Consent for Publication. Not applicable.

Availability of data and materials. The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

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