Novel LAMP-2 Mutation in a Family With Danon Disease Presenting With Hypertrophic Cardiomyopathy

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Danon disease is an X-linked dominant multisystem disorder that includes hypertrophic cardiomyopathy with skeletal myopathy, and results from mutations in the gene encoding the lysosome-associated membrane protein-2 (LAMP-2). To date, over 20 different mutations in *LAMP2* have been identified. Three members of a family, a male proband (18 years old) and 2 sisters (15 and 20 years old) were studied. Their mother had been diagnosed with dilated cardiomyopathy at the age of 39 years, and died from advanced heart failure at the age of 43 years. The proband developed marked concentric hypertrophy at the age of 5 years and DNA analyses revealed a novel hemizygous frameshift mutation (c.573delA) in exon 5. The 2 affected sisters were also heterozygous for the same mutation. Functional analyses of this novel *LAMP2* mutation are mandatory.

Key Words: Cardiomyopathy; Genetics; Heart failure

Hypertrophic cardiomyopathy (HCM) is a genetic cardiac disease characterized by cardiac hypertrophy and increased frequency of premature death. However, mutations in the known contractile protein genes are confirmed in, at most, one-third of cases[1] Recent molecular studies of HCM patients without sarcomeric protein mutations have revealed other genetic causes of cardiac hypertrophy; for example, a metabolic cardiomyopathy because of accumulation of glycogen, thereby leading to severe left ventricular hypertrophy (LVH)[2] Danon disease is an X-linked dominant multisystem disorder that includes HCM with skeletal myopathy, and affected patients have mutations in the gene encoding the lysosome-associated membrane protein-2 (LAMP-2)[3] Affected males typically develop cardiac symptoms before the age of 20 years, whereas most affected women develop cardiomyopathy in adulthood[4] To date, more than 20 mutations in *LAMP2* have been identified[2,3,5,6–13] with most resulting in loss of a functional protein. Mice lacking LAMP-2 show similar features to patients, including cardiac hypertrophy and dysfunction[14,15]

We report a novel *LAMP2* mutation in members of a family with Danon disease, who had been diagnosed as having familial HCM.

(Received March 7, 2008; revised manuscript received April 14, 2008; accepted April 23, 2008; released online December 5, 2008)
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Case Reports

The proband is a 17-year-old boy who was admitted for diagnosis of the etiology of left ventricular (LV) dysfunction (Fig 1). However, when he was 5 years old, he had been referred to another hospital because of heart murmur and was diagnosed as having obstructive HCM. Laboratory tests showed elevation of serum AST, ALT, LDH and aldolase, suggesting glycogen storage disease or Fabry disease, but the activity of both acid maltase and α-galactosidase was confirmed to be within the normal range.

On examination, he had normal intelligence. Neurologi-

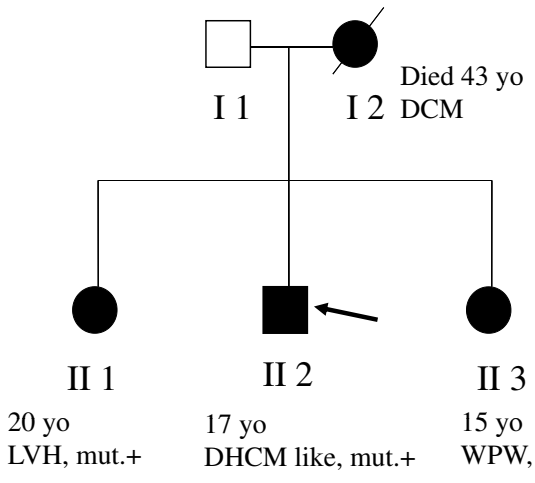

Fig 1. Pedigree of the family with *LAMP2* mutations. Black symbols indicate affected subjects, white symbol indicates unaffected subject. The proband is indicated by an arrow. Slashed symbol indicates the deceased member. mut.+, *LAMP2* mutation documented in the present study; DCM, dilated cardiomyopathy; LVH, left ventricular hypertrophy; DHCM, dilated hypertrophic cardiomyopathy; WPW, Wolff-Parkinson-White syndrome.

cal examination revealed mild skeletal myopathy. Electrocardiogram (ECG) showed bizarre QRS waves, widening of the QRS complex and ST changes (Fig 2). No significant premature ventricular contractions were observed on 24-h Holter monitoring. Echocardiography revealed marked LVH and progressive LV dilatation (Fig 3), a finding compatible with the dilated phase of HCM. The combined characteristics of HCM and myopathy led us to investigate Danon disease as the underlying etiology. Skeletal muscle biopsy showed mild variation in fiber size (Fig 4A) and small vacuoles were seen in several fibers, which appeared as basophilic granules rather than vacuoles in the hematoxylin–eosin preparation (Fig 4B). Acetylcholine and nonspecific esterase activities were associated with the vacuolar membranes (Figs 4C,D). These findings supported the diagnosis of Danon disease. Retinal lesion, which is also reported to be a feature of Danon disease,[16] was not observed in the proband.

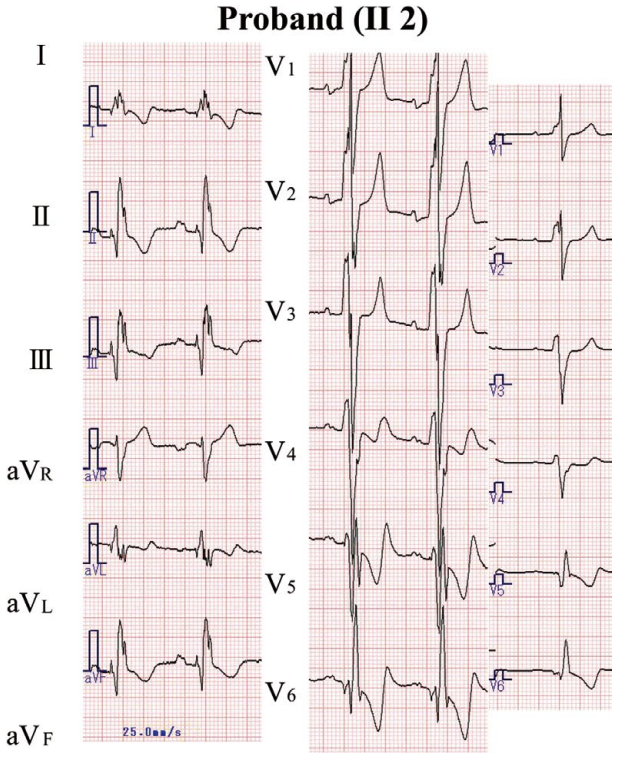

Fig 2. Electrocardiogram of the proband aged 17 years shows bizarre QRS waves, widening of QRS complex and ST changes.

His mother and 2 sisters were also diagnosed with cardiac disease (Fig 1). His mother died at the age of 43 years from advanced heart failure, 4 years after the onset of dilated cardiomyopathy. His elder sister had abnormal ECGs and mild LVH on echocardiography, and the younger sister had electrocardiographic abnormalities suggestive of Wolff-Parkinson-White syndrome, and LVH on echocardiography. No significant premature ventricular contractions were observed on 24-h Holter monitoring in either sister.

Genetic studies were carried out after informed consent was given by all participating patients, as approved by the institutional review board. In the probands, a hemizygous c.573delA variant was identified in exon 5 of *LAMP2* (Fig 5). This deletion results in a frameshift and a premature stop codon (p.D192TfsX49). Both of his sisters were heterozygous for this deletion (Fig 5), but his father did not carry the mutation. DNA was not available from their deceased mother, but as this is an X-linked gene it is likely that the mother was a carrier.

Discussion

Danon disease is an X-linked dominant multisystem disorder that includes HCM with skeletal myopathy. The patients have mutations in the gene encoding LAMP-2, which is a principal protein of the lysosomal membrane[3] It has been suggested that the lysosomal membrane is responsible for transport of degradation products to the cytoplasm and regulation of fusion events between lysosomes them-

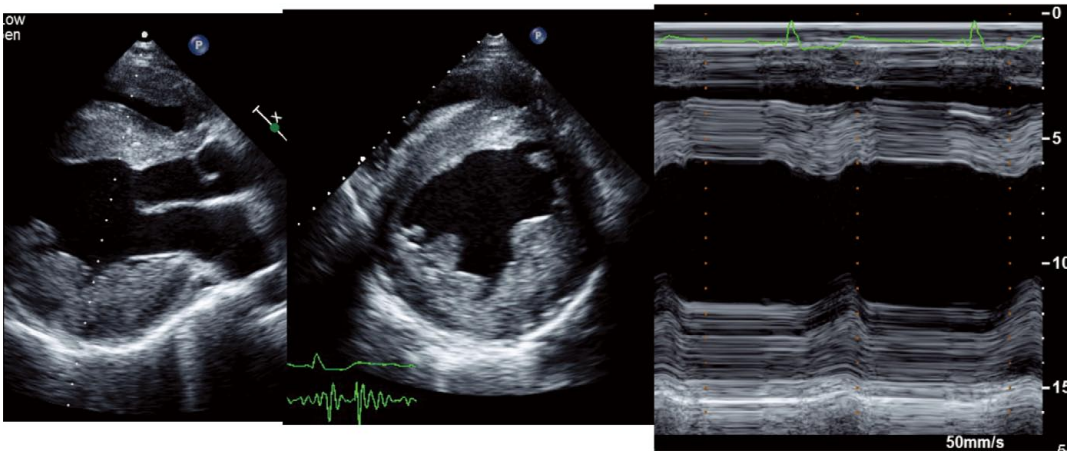

Fig 3. Echocardiographic assessment of the proband. Parasternal long-axis view, M mode and short-axis view show marked concentric hypertrophy with dilatation of the left ventricle (70 mm at end-diastole). The interventricular septum is 22 mm thick and the left ventricular posterior wall is 23 mm. Wall motion of the left ventricle is depressed, as shown by a decrease in the ejection fraction (0.42). These findings are suggestive of the dilated phase of hypertrophic cardiomyopathy.

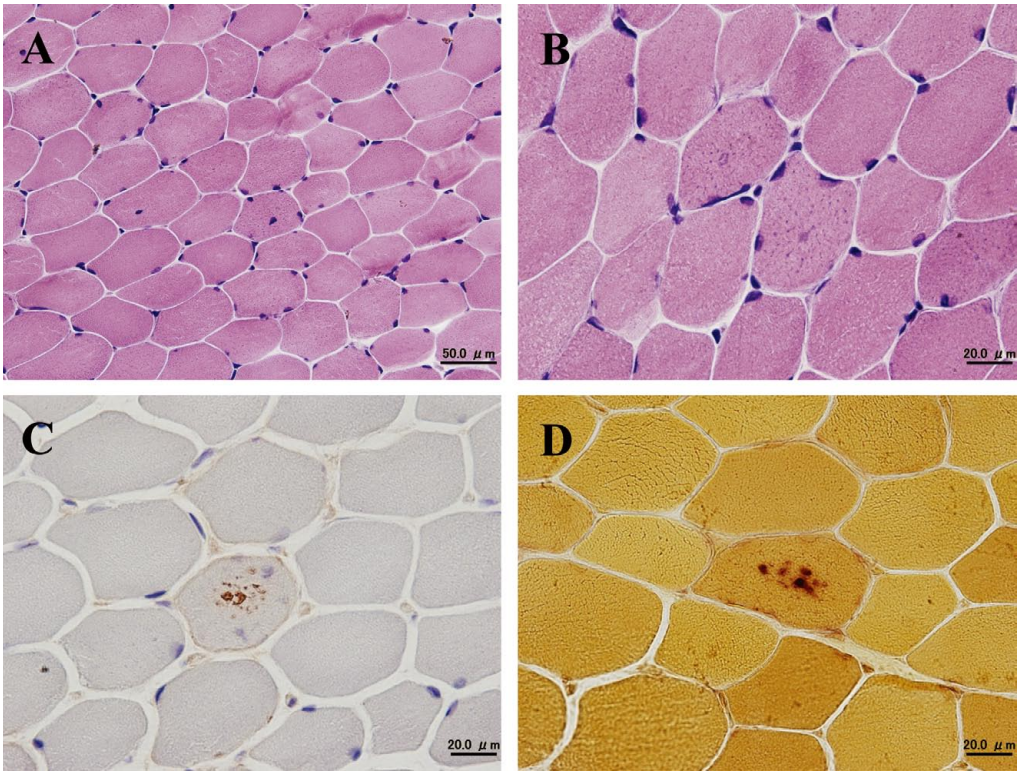

Fig 4. Histochemical analysis of skeletal muscle samples from the biceps of the proband. (**A**) Mild variation in fiber size and there are several fibers with tiny basophilic intracytoplasmic vacuoles scattered throughout (**B**). The vacuolar membrane has high activities of acetylcholine esterase (**C**) and nonspecific esterase (**D**). (**A**,**B**) Hematoxylin and eosin stain; (**C**) acetylcholine esterase; (**D**) nonspecific esterase.

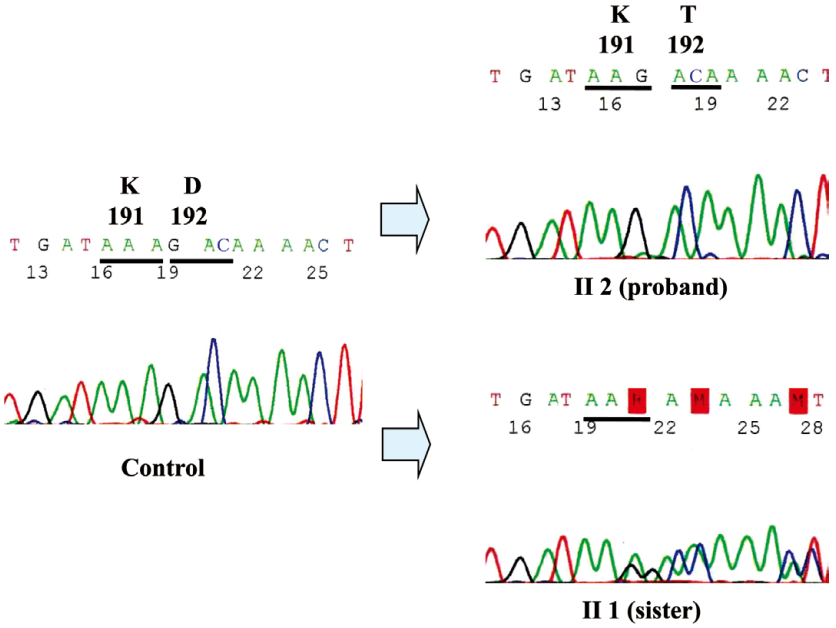

Fig 5. Detection of the 573delA by DNA sequence analysis of exon 5 of *LAMP2*. The DNA sequence from the proband (II 2) shows the hemizygous deletion, and that from elder sister (II 1) shows the heterozygous deletion.

selves and other organelles![7] Accumulation of autophagic vacuoles has been documented in the cardioskeletal muscles of both patients with Danon disease and in LAMP-2-deficient mice[3,14] Thus, LAMP-2 could have a pivotal role in the degradation of autophagosomes within the lysosomal compartment.

LAMP2 is located on chromosome Xq24-q25. It consists of 9 exons in humans, comprising a large luminal domain encoded by exons 1–8 and 2 small transmembrane and cytoplasmic domains encoded by exon 9. To date, at least 27 different mutations leading to LAMP-2 deficiency have been identified in unrelated cases with similar clinical fea-

Table 1 Previous Reports of *LAMP2* Mutations in Danon Disease

Authors	*Year*	*Reference*	*Mutation*	*Location of LAMP2 mutation*	*Effect on mRNA*
Nishino	*2000*	*3*	*2-bp del*	*Exon 9b*	*Frame shift*
			T440A	*Exon 4*	*Nonsense*
			IVS6+5G→C	*Intron 6*	*E6 skipping*
			T974AA	*Exon 8*	*Frame shift*
			C813G	*Exon 8*	*Nonsense*
			IVS5+1G→A	*Intron 5*	*6-bp insertion*
			10-bp deletion	*Intron 5/Exon 6 junction*	*10-bp deletion*
			14delG	*Exon 1*	*Frame shift*
Takahashi	*2002*	*7*	*883insT*	*Exon 7*	*Frame shift*
Horvath	*2003*	*8*	*IVS7→1G*	*Exon 8*	*Abnormal splicing?*
Charron	*2004*	*9*	*520C→T*	*Exon 4*	*Nonsense*
			173-179del	*Exon 1*	*Frame shift*
Arad	*2005*	*2*	*Unspecified*		*Nonsense*
			IVS1+1G→T	*Intron 1*	*Abnormal splicing*
			IVS1-2A→G	*Intron 1*	*Abnormal splicing*
			IVS6+1_delGTGA	*Intron 6*	*Abnormal splicing*
			IVS6-2A→G	*Intron 6*	*Nonsense decay*
			928A→G		*Abnormal splicing*
Yang	*2005*	*5*	*1075C→T*	*Exon 8*	*Nonsense*
			467T→G	*Exon 4*	*Nonsense*
Lobrinus	*2005*	*10*	*470C→G*	*Exon 4*	*Nonsense*
Balmer	*2005*	*11*	*138G→A*	*Exon 2*	*Nonsense*
Echaniz-Laguna	*2006*	*12*	*102_103delAG*	*Exon 2*	*Frame shift*
Fanin	*2006*	*6*	*796-797insC*	*Exon 6*	*Frame shift*
			680-701del	*Exon 7*	*Frame shift*
			294G→A	*Exon 3*	*Nonsense*
Taylor	*2007*	*13*	*1219delA*	*Exon 8*	*Frame shift*
Dougu	*2009*		*573delA*	*Exon 5*	*Frame shift*

Abnormal splicing means the mutation is predicted to affect mRNA splicing but it is not proven. LAMP2, lysosome-associated membrane protein-2.

tures (Table 1). The novel mutation identified in this family is encoded in exon 5 and leads to premature truncation of LAMP-2 with abolition of the transmembrane domain, and thus can lead to an accumulation of autophagic vacuoles mainly in cardiac and skeletal muscle cells.

The prognosis for patients with Danon disease is poor, especially males[4] In females, signs of heart failure could emerge after 40 years. These findings are compatible with the clinical course of the family presented here. In the proband, signs of heart failure were not evident before 17 years of age, but emerged at 18 years. His mother suffered heart failure at 39 years and died at 43 years. Thus, patients with Danon disease need to be followed closely and cardiac transplantation is the treatment of choice.

Functional analysis of the mutation found in the present family has not been performed, but frameshift mutations are likely to lead to the absence of functional protein (males) or haplo-insufficiency (females), as previously demonstrated?[2–5]

Acknowledgments

We thank Drs Neil E. Bowles and Zhao Yang for their helpful comments and suggestions.

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