Case Report

Early onset of cardiomyopathy and intellectual disability in a girl with Danon disease associated with a de novo novel mutation of the LAMP2 gene

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Danon disease, primary lysosome-associated membrane protein-2 (LAMP-2) deficiency, is characterized clinically by cardiomyopathy, myopathy and intellectual disability in boys. Because Danon disease is inherited in an X-linked dominant fashion, males are more severely affected than females, who usually have only cardiomyopathy without myopathy or intellectual disability; moreover, the onset of symptoms in females is usually in adulthood. We describe a girl with Danon disease who presented with hypertrophic cardiomyopathy and Wolff-Parkinson-White (WPW) syndrome at 12 years of age. Subsequently, she showed signs of mild learning disability and intellectual disability on psychological examinations. She had a de novo novel mutation in the *LAMP-2* gene and harbored an identical c.749C > A (p.Ser250X) variant, resulting in a stop codon in exon 6. She showed decreased, but not completely absent LAMP-2 expression on immunohistochemical and Western blot analyses of a skeletal muscle biopsy specimen, which has been suggested to be caused by a 50% reduction in LAMP-2 expression (LAMP-2 haploinsufficiency) in female patients with Danon disease caused by a heterozygous null mutation. To our knowledge, our patient is one of the youngest female patients to have been given a diagnosis of Danon disease. In addition, this is the first documented case in a girl that was clearly associated with intellectual disability, which is very rare in females with Danon disease. Our findings suggest that studies of female patients with Danon disease can extend our understanding of the clinical features of this rare disease.

Key words: autophagic vacuoles, cardiomyopathy, Danon disease, intellectual disability, lysosome-associated membrane protein-2 (LAMP-2).

INTRODUCTION

Danon disease, an X-linked cardioskeletal myopathy, is caused by primary deficiency of lysosome-associated membrane protein-2 (LAMP-2).[1,2] The disease is characterized clinically by the triad of cardiomyopathy, myopathy, and intellectual disability in males, but only cardiomyopathy in females.[1–5] Skeletal muscle biopsies have revealed autophagic vacuoles with sarcolemmal features (AVSF) in the muscle fibers of male patients.[6] Cardiac involvement, especially hypertrophic cardiomyopathy and Wolff-Parkinson-White (WPW) syndrome, is the most prominent and consistent manifestation of both male and female patients with Danon disease and is diagnostically very important. However, female patients with Danon disease have a relatively mild clinical phenotype. We previously reported that the mean age at presentation of the disease was 38 ± 12 years in females, as compared with 17 ± 7 years in males.[2]

We describe a 15-year-old girl with Danon disease who presented with early-onset of hypertrophic cardiomyopathy and mild intellectual disability. She had a de novo novel mutation, c.749C > A (p.Ser250X), in the *LAMP-2* gene, and her muscles showed decreased LAMP-2 expression. To our knowledge, our patient is one of the youngest female patients to be have been given a diagnosis of Danon disease; moreover, the onset of symptoms in females is usually in adulthood. In addition, this is the first documentation of a girl with Danon disease clearly associated with intellectual

disability, which is very rare among females with Danon disease. Our findings suggest that studies of female patients with Danon disease can extend our understanding of the clinical features of this rare disease.

CLINICAL SUMMARY

A 12-year-old Japanese girl was referred to our hospital because of an abnormal electrocardiographic pattern detected on a physical examination at her school. She recently had noted mild fatigue after effort. In addition, she was indicated as having mild learning disturbance. She was born at full term and had no neonatal problems. Her growth and developmental milestones were generally normal. Her family members were all healthy without any laboratory abnormalities. Her mother and her older brother had normal cognitive status and muscle strength. The results of a cardiac evaluation were also normal.

On physical examination, she was slender with no dysmorphic features. A systolic murmur was unremarkable. Neurological examination revealed normal muscle strength and normal deep-tendon reflexes. On laboratory examinations, the serum creatine kinase level was 55 IU/L (normal range: 30–160), and serum levels of brain natriuretic peptide and Troponin T were slightly elevated. The results of screening for Pompe's disease with dried blood spots collected on filter paper were negative. The chest X-ray film showed mild cardiomegaly. WPW syndrome was diagnosed by electrocardiography. Transthoracic echocardiogram showed asymmetrical left ventricular (LV) hypertrophy with an end-diastolic interventricular septum dimension of 9.8 mm (145% of normal subjects (NS) at this age), a LV posterior wall dimension of 6.6 mm (91% of NS) and an end-diastolic LV diameter of 36.3 mm (71% of NS). Systolic function was preserved, with an ejection fraction of 77%. Late gadolinium enhancement on cardiac MRI showed focal hyperenhancement at the apical region of the LV (Fig. 1), as previously described.[7] MRI of the brain, 123I-iomazenil single photon emission computed tomography (SPECT), and electroencephalography revealed no remarkable abnormalities. On psychological examination, she had mild learning disability and intellectual disability with a full-scale intelligence quotient (IQ) of 78 (verbal comprehension index, 72; perceptual reasoning index, 76) on the Wechsler Intelligence Scale for Children-IV (WISC-IV). However, her learning disturbance did not progress, and her condition remained stable. She also had decreased visual acuity. An ophthalmologic examination revealed mild myopia with retinopathy.

For sequence analysis, DNA was extracted from frozen muscles with phenol/chloroform. We sequenced the entire coding region, including the exon/intron junctions of the *LAMP-2* gene, as previously described.[1,2] We identified a novel nonsense mutation in the *LAMP-2* gene in the patient.
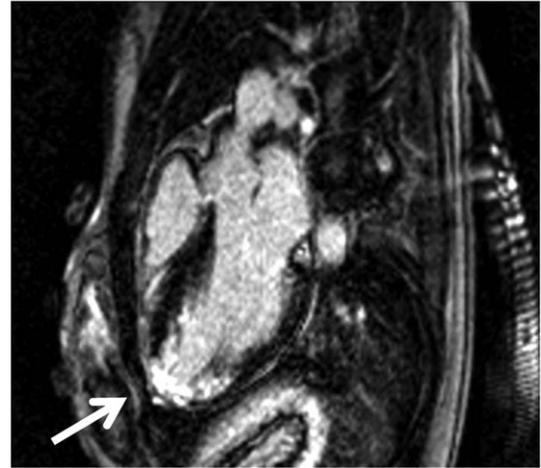

Fig. 1 Cardiac MRI. Late gadolinium enhancement on cardiac MRI in the two-chamber view showed focal hyperenhancement at the apical region of the left ventricle (arrow).

She harbored an identical c.749C > A (p.Ser250X) variant, resulting in a stop codon in exon 6 (Fig. 2). Her mother had no *LAMP-2* gene mutation, and her older brother was healthy, indicating the mutation was de novo. On screening 100 Japanese controls, the same mutations were not found.

PATHOLOGICAL FINDINGS

Biopsy specimens were taken from the patient's left biceps brachii muscle. The specimens were frozen in liquid nitrogen-cooled isopentane for histochemical analysis and fixed in buffered glutaraldehyde for electron microscopy. Ten-micrometer-thick transverse frozen sections were stained with HE and a battery of histochemical methods. In addition, 6 μm-thick cryosections were stained with mouse monoclonal antibodies, as previously described.[8] The sections were incubated at 37°C with primary mouse IgG antibodies against LAMP-2 (H4B4, Developmental Studies Hybridoma Bank, Iowa City, IA, USA), the C-terminal of dystrophin (Novocastra, Newcastle-Upon-Tyne, UK), laminin α2 (Novocastra), α-sarcoglycan (Novocastra) and LC3 (Proteintech Group, Rosemont, IL, USA). The sections were subsequently incubated at room temperature with a secondary antibody, goat anti-mouse IgG (Leinco, St. Louis, MO, USA). Control specimens were obtained from five patients with morphologically normal muscle.

The patient's muscle biopsy specimens showed mild variations in fiber size, but no small vacuoles. Acetylcholinesterase and non-specific esterase staining appeared normal. Immunohistochemical reactivity for LAMP-2 was not absent, but was weaker than that in normal controls (Fig. 3). The other immunohistochemical findings, including LC3, showed no abnormalities and no AVSF.
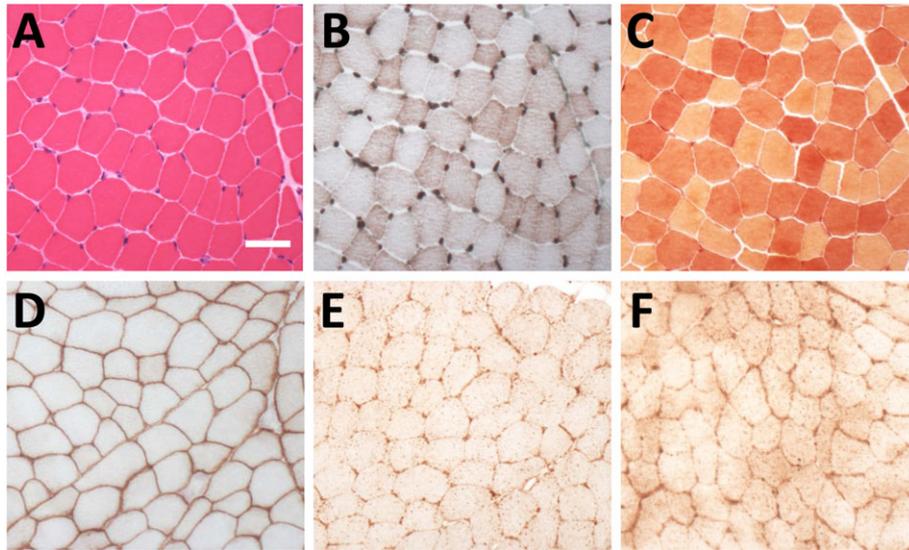
Fig. 2 Light microscopic findings of femoral muscle specimens. Transverse sections of skeletal muscle biopsy specimens from the girl with Danon disease (A E) and female controls (F). Histochemical analyses for HE (A), acetylcholinesterase (B) and non-specific esterase stain (C). She showed mild myogenic changes and no vacuoles in muscle fibers. Immunohistochemical analyses for dystrophin (D) and lysosome-associated membrane protein-2 (LAMP-2) (E, F). LAMP-2 is decreased in muscle of the affected girl, but is present in female control muscles. Bar 50 μm.
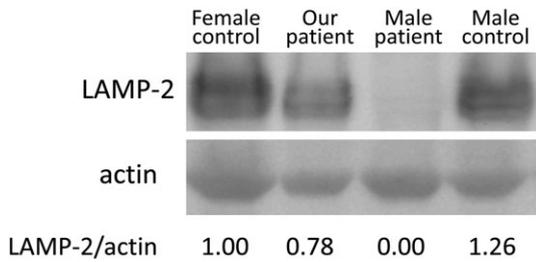

Fig. 3 Western blot analysis. Skeletal muscle extracts from a male control, a female control, our affected girl with Danon disease and a male control with Danon disease were blotted and labeled with antibodies against lysosome-associated membrane protein-2 (LAMP-2) and β-actin. LAMP-2 is present in both the male and female control muscles, but is decreased in the girl's muscle and is absent in the male patient's muscle. Of note, the LAMP-2 protein level in the patient was equivalent to 78% of that in the female controls, most likely because of 'LAMP-2 haploinsufficiency'.

We performed Western blot analysis on skeletal muscle specimens from the patient, a male disease control, and male and female controls, as previously described.[5] Frozen tissue specimens were briefly washed with cold PBS, homogenized in triple-detergent lysis buffer, and spun down. The supernatant was collected and stored at −80°C until use. We separated 5-μL samples by electrophoresis and electrotransferred the proteins onto nitrocellulose membranes. The membranes were overlaid with a monoclonal antibody against LAMP-2 (H4B4). Quantitative measurements were obtained by densitometry and expressed as a ratio of the values of LAMP-2 to β-actin. On Western blot analysis,

LAMP-2 was present nearly equally in muscle from both the male and female controls. In contrast, LAMP-2 was undetectable in the male disease control, whereas LAMP-2 protein level in the patient was equivalent to 78% of the level in the female controls (Fig. 4).
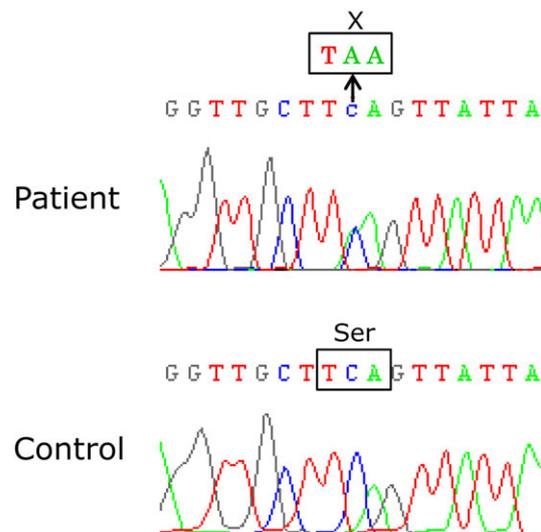

Fig. 4 Genetic analysis for lysosome-associated membrane protein-2 (*LAMP-2*) gene. A novel nonsense mutation, c.749C > A (p.Ser250X), was identified in the patient. Her mother had no *LAMP-2* gene mutation.

DISCUSSION

We have described a girl with genetically confirmed Danon disease. She presented with hypertrophic cardiomyopathy and WPW syndrome at 12 years of age, followed by the onset of mild intellectual disability, but no clinical evidence of a skeletal myopathy. Genetic analysis identified a de novo, novel nonsense mutation in the *LAMP-2* gene.

Our female patient showed decreased, but not absent LAMP-2 expression on immunohistochemical and Western blot analyses of a skeletal muscle biopsy specimen, as we reported for the first time previously in a different female patient. We attribute these findings to a 50% reduction in LAMP-2 expression (LAMP-2 haploinsufficiency) caused by a heterozygous null mutation in the *LAMP-2* gene in heterozygous female patients with Danon disease.[5] Actually, our results suggest that 50% LAMP-2 expression may prevent the development of skeletal muscle symptoms, but not cardiac symptoms. Female patients with Danon disease have a relatively milder and later-onset clinical phenotype than male patients.[1,2] As the underlying reason, we suggest that haploinsufficiency along with skewed X-chromosome inactivation may be clinically significant determinants of phenotype in heterozygous female patients with Danon disease. However, data on tissue LAMP-2 expression in female patients remain limited.

Interestingly, to our knowledge, our girl patient is one of the youngest female patients to be have ever given a diagnosis of Danon disease, symptoms of which usually develop in adulthood in females. Very few female patients with disease onset in childhood have been previously described.[9–11] However, these female patients showed different phenotypes, particularly with regard to the severity of cardiomyopathy. As for cardiomyopathy, Oldfors *et al.* reported that an uneven distribution of LAMP-2 protein might cause deleterious effects in the myocardium.[9] In contrast, Maron *et al.* reported that many autophagic vacuoles were scattered in the myocardium.[10] In addition, we previously reported that vacuolated fibers in skeletal muscle increase with age in Danon disease. These findings suggest that the progression of clinical symptoms is related not only to primary LAMP-2 deficiency, but also to increased numbers of small vacuoles, and that the accumulation of autophagic materials is likely to contribute to pathogenesis of the disease. Therefore, we hypothesize that the expression of clinical symptoms depends not only directly on the deficiency of LAMP-2, but also on the accumulation of autophagic material in vacuoles.

Another interesting finding was that our girl patient showed mild intellectual disability, which is very rare in females with Danon disease. To our knowledge, girls with Danon disease associated with intellectual disability diagnosed on detailed neurological and psychological examinations have not been previously described. We believe that this is the first documented case of intellectual disability in a young girl with Danon disease. In our previous study, mild mental retardation was confirmed in 30% of male patients with Danon disease. In contrast, intellectual disability in female patients with Danon disease has been reported to be very rare. In fact, in our previous study, only one (6%) of 18 women with Danon disease had mental retardation.[2,12] Boucek *et al.* reported that mild and nonspecific cognitive complaints were noted in adult female patients (7/15, 47%), but did not describe any neurological or psychological details.[13]

The pathomechanism of intellectual disability remains unclear and unestablished in Danon disease, because there are limited data regarding LAMP-2 expression in tissues of female patients, especially in the brain. In our previous study, electroencephalography showed mild abnormalities in two male patients. One male patient showed evidence of decreased cerebral glucose metabolism in the cerebral cortex on positron emission tomography.[14] There were no CNS manifestations, and no patient showed structural brain abnormalities on MRI or SPECT, consistent with our findings in the present patient. In two autopsy cases, we found vacuolar changes in the cytoplasm of the red nucleus.[4] Recently, we have reported lysosomal storage and advanced senescence in the brain of a male patient with Danon disease.[15,16] However, it remains unknown whether these abnormalities directly account for the intellectual disability.

In conclusion, to our knowledge, our patient is one of the youngest female patients to be have been given a diagnosis of Danon disease, symptoms of which usually develop in adulthood in females. In addition, this is the first documented case in a girl that was clearly associated with intellectual disability, which is very rare in females with Danon disease. The current findings suggest that studies of female patients with Danon disease can extend our understanding of the clinical features of this rare disease. Most cases of Danon disease in female patients are diagnosed when male probands with the disease are evaluated, and very few female patients with no family history of the disease have been documented, indicating that patients such as ours are very rare. We suggest that asymptomatic female relatives of patients with Danon disease should be screened for cardiomyopathy and closely followed up to detect early signs of a potentially life-threatening condition. Moreover, the correlation between genotype and phenotype, including intellectual disability and retinopathy, has yet to be established in Danon disease without mild symptoms in patients who have a *LAMP-2* gene mutation in exon 9. We suspect that genetic factors, including genotype–phenotype correlation, might be one of the important factors contributing to the unusual presentation of clinical features in our female patient. The exact mechanisms leading to the different phenotypes in Danon disease remain to be further investigated.

ACKNOWLEDGMENTS

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

K. Sugie was responsible for the overall study design, participated in the organization, planning and coordination of the study, and wrote the manuscript. H. Yoshizawa, K. Onoue, Y. Nakanishi, N. Eura, M. Ogawa, T. Nakano, Y. Sakaguchi, YK. Hayashi, T. Kishimoto, M. Shima, Y. Saito, I. Nishino, and S. Ueno contributed to running the study and analyzed and interpreted the data.

CONSENT

Written informed consent for publication of this case report was obtained from the patient. A copy of the written consent form is available for review from the Editor of this journal.

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