Identification of *LAMP2* mutations in early-onset hypertrophic cardiomyopathy by targeted exome sequencing

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X-linked dominant mutations in lysosome-associated membrane protein 2 (*LAMP2*) gene have been shown to be the cause of Danon disease, which is a rare disease associated with clinical triad of cardiomyopathy, skeletal myopathy, and mental retardation. Cardiac involvement is a common manifestation and is the leading cause of death in Danon disease. We report a case of a 24-month-old boy with hemizygous *LAMP2* mutation who presented with failure to thrive and early-onset hypertrophic cardiomyopathy. We applied targeted exome sequencing and found a novel hemizygous c.692del variant in exon 5 of the *LAMP2* gene, resulting a frameshift mutation p.Thr231Ilefs*11. Our study indicates that target next-generation sequencing can be used as a fast and highly sensitive screening method for inherited cardiomyopathy.

Key words: Lysosomal-associated membrane protein 2, Danon disease, Hypertrophic cardiomyopathy, Whole exome sequencing.

Introduction

Danon disease is a rare X-linked dominant disorder, initially described in two boys presenting with cardiomyopathy, skeletal myopathy, and mental retardation [1]. This disease is caused by mutations in lysosome-associated membrane protein 2 (*LAMP2*) gene located at Xq24 [2]. Major clinical findings include skeletal and cardiac myopathy, cardiac conduction abnormalities, intellectual difficulties, and retinal involvement [3]. Cardiac involvement is the leading cause of death in patients with Danon disease [4]. Early diagnosis is important for family counseling, but molecular diagnosis is difficult because of genetic heterogeneity of cardiomyopathy. With advances of next-generation sequencing (NGS), massive parallel sequencing of multiple genes facili-

tates confirmative genetic approach to patients with cardiomyopathy [5-7]. We report the case of a 24-month-old boy with hemizygous *LAMP2* mutation who presented with failure to thrive and early-onset of hypertrophic cardiomyopathy (HCMF).

Case

A 24-month-old boy visited our endocrinology clinic, concerned with short stature and poor weight gain. His height was 78.9 cm (−2.03 standard deviation score [SDS]) and his weight was 9.2 kg (−2.66 SDS). He was born at 38 weeks gestation with 2.64 kg (−1.71 SDS) from non-consanguineous and healthy parents. He had an admission history of neonatal intensive care unit for transient tachypnea at newborn. On physical examina-

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Conflict of interest: The authors declare that they do not have any conflicts of interest.

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tion, there was no evidence of muscle weakness and dysmorphic morphology. His development was normal. Laboratory findings including white blood cell of $10,100/\text{mm}^3$, hemoglobin of 13.6 g/dL , platelets of $360,000/\text{mm}^3$, thyrotropin stimulating hormone of $2.05\text{ }\mu\text{IU/mL}$, and free T4 of 1.54 ng/dL were unremarkable, but elevated levels of aspartate aminotransferase (161 U/L) and alanine aminotransferase (88 U/L) were noted. Viral studies such as hepatitis virus A, hepatitis virus B, hepatitis virus C, Epstein-Barr virus, and cytomegalovirus were negative. Elevated liver enzymes persisted and elevated creatinine kinase (337 U/L) was remarked. So he was referred to the pediatric neurology clinic to evaluate neuromuscular disease.

He had motor grade of 5 in all limbs with normal muscle mass and muscle tone. The deep tendon reflex was normal and there was no Gower's sign. He was able to walk independently in a normal gait. His neurology examination revealed no special findings. Developmental milestones in all categories were not delayed, including gross and fine motor milestones. A harsh grade 2 systolic murmur was denoted on the apex area of left anterior chest with regular heartbeat, therefore he was consulted to the pediatric cardiology clinic. The electrocardiogram showed biventricular hypertrophy with high voltage on every chest lead. Echocardiography showed thickened posterior wall of left ventricle and septum with normal systolic function and diastolic dysfunction (Fig. 1).

We initially performed metabolic assessment to distinguish inherited metabolic cardiomyopathy including fatty acid oxidation disorders and Pompe disease. However, serum amino acid analysis, urine organic acid analysis, acylcarnitine profiles, and molecular analysis of *GAA* gene results were all unremarkable. Then, we applied targeted exome sequencing to identify monogenic disorders of congenital cardiomyopathy using the MiSeq platform (Illumina, Inc., San Diego, CA, USA) and the TruSight One Sequencing panel (Illumina, Inc.). This panel contains multiple cardiomyopathy-related genes that have been previously reported to be associated with HCMP; the genes related with sarcomere proteins, the genes related with inherited left ventricular hypertrophy, the genes related with syndromic disease with cardiomyopathy, the genes related with metabolic cardiomyopathy, and the genes related with neuromuscular disorders (Table 1). Using these analyses, we identified a novel hemizygous for c.692del variant in exon 5 of the *LAMP2* gene, resulting in a frameshift mutation p.Thr231Ilefs*11 (Fig. 2). His mother was not a carrier of the mutation. No retinal abnormalities were detected at ophthalmic examination.

Discussion

This case illustrated the clinical efficacy of targeted exome sequencing in patients with HCMP. Usually, Danon disease is presented with cardiomyopathy, skeletal myopathy and varying degree of intellectual disabilities, however in our case, the patient only manifested with early-onset HCMP. Although this condition is a syndromic disease, it is not easy to approach the diagnosis if only one clinical symptom is developed. Therefore, NGS can be an effective diagnostic tool of HCMP.

Cardiomyopathy in Danon disease is progressive, and it develops early as a hypertrophic phenotype. The age at cardiac presentation of Danon disease varied from infancy to the second decade of life in men, and sudden death may occur in patients with this disease in the second or third decade of life. Also, affected males have learning disability or cognitive problems in

Fig. 1. (A) Hypertrophy of septum (arrows) and posterior wall of left ventricle (arrowheads) by subcostal four chamber view by echocardiography. (B) Thickened septum and posterior wall of left ventricle with normal systolic function.

Table 1. Congenital cardiomyopathy gene list included in current panel

Etiology	Gene symbol	Description
Hypercardiomyopathic sacromere proteins	<i>MYH7</i>	β -Myosin heavy chain
	<i>MYBPC3</i>	Cardiac myosin-binding protein C
	<i>TNNT2</i>	Cardiac troponin T
	<i>TNNI3</i>	Cardiac troponin I
	<i>TPM1</i>	α -Tropomyosin
	<i>MYL2</i>	Regulatory myosin light chain
	<i>MYL3</i>	Essential myosin light chain
	<i>ACTC1</i>	α -Cardiac actin
	<i>ACTN2</i>	α -Actinin 2
	<i>MYH6</i>	Myozenin-2
	<i>TCAP</i>	Telethonin
	<i>TNNC1</i>	Troponin C, slow skeletal and cardiac muscles
	<i>PLN</i>	Cardiac phospholamban
	<i>MYOZ2</i>	Myozenin-2
	<i>NEXN</i>	Nexilin
Inherited left ventricular hypertrophy	<i>CSRP3</i>	Muscle LIM protein
	<i>PRKAG2</i>	AMP-activated protein kinase mutation, WPW syndrome
	<i>LAMP2</i>	Danon disease
Multiple congenital anomaly syndrome	<i>PTPN11</i>	Noonan syndrome, LEOPARD syndrome
	<i>H19</i>	Beckwith-Wiedemann syndrome
	<i>KCNQ1</i>	Beckwith-Wiedemann syndrome
	<i>CDKN1C</i>	Beckwith-Wiedemann syndrome
	<i>HRAS</i>	Costello syndrome
	<i>CREBBP</i>	Rubinstein-Taybi syndrome
	<i>BRAF</i>	Cardiofaciocutaneous syndrome
Inborn errors of metabolism	<i>GAA</i>	Pompe disease
	<i>IDUA</i>	Hurler syndrome (mucopolysaccharidosis type I)
	<i>IDUA</i>	Scheie syndrome
	<i>GLA</i>	Fabry disease
	<i>MAN2B1</i>	Mannosidosis
	<i>MUT</i>	Methylmalonic aciduria
Mitochondrial disorders	<i>NDUFS4</i>	Leigh syndrome, complex I deficiency
	<i>NDUFA2</i>	Leigh syndrome, complex I deficiency
	<i>NDUFS3</i>	Leigh syndrome, complex I deficiency
	<i>NDUFS7</i>	Leigh syndrome, complex I deficiency
	<i>NDUFA2</i>	Leigh syndrome, complex I deficiency
	<i>ACADVL</i>	Very long chain acyl-coenzyme A dehydrogenase deficiency
	<i>HADHA</i>	Long chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency
Neuromuscular disorders	<i>FXN</i>	Friedreich ataxia
	<i>DMPK</i>	Myotonic dystrophy
	<i>RYR1</i>	Minicore (multicore) myopathy

LIM, lin-11, islet-1, mec-3; AMP, adenosine monophosphate; WPW, Wolff-Parkinson-White; LEOPARD, lentiginos, electrocardiographic conduction defects, ocular hypertelorism, pulmonary stenosis, abnormalities of genitalia, retardation of growth, and deafness.

70–100% [3,4]. Skeletal myopathy is also cardinal feature, which denotes progressive proximal muscle weakness of the shoulders, neck, and legs in 80–90% of males [4]. The therapeutic option

for Danon disease was limited, but cardiac transplantation enhances the survival of patients [8].

Since the outcome of HCMP in childhood varies by its under-

Fig. 2. Pedigree of the patient and the partial DNA sequence of *LAMP2* gene of patient and his mother; novel hemizygous c.692del variant (p.Thr231Ilefs*11) in exon 5 of the *LAMP2* gene.

lying disease and age at diagnosis, early detection and diagnosis is important [9]. HCMP in childhood is genetically heterogeneous with many different genes [10]. It can also manifest systemic features affecting non-cardiac organs. Because of these diverse causes, genetic testing is recommended with high level of evidence along with the metabolic work-up in many guidelines [11,12]. In previous studies, 40–60% of the cause for HCMP was mainly mutations in cardiac sarcomere protein genes using single gene or multiple panel testing by Sanger sequencing [11,13,14]. Traditional method to diagnose Danon disease was to confirm the histological and immunohistochemistry findings, such as vacuolation of muscle fibers and absence of LAMP2 protein, respectively, through the muscle biopsy. Recently, NGS has been an advanced diagnostic tool to evaluate the cause of cardiomyopathy. Not only it reduced the turn-around-time compared to the traditional method, but it also increased the diagnostic yield, and enabled to avoid invasive technique necessary for diagnosis such as skeletal/cardiac muscle biopsy [6,15]. Recent reports on congenital cardiomyopathy including Danon disease using the NGS method are increasing [7].

To date, more than 90 different disease-causing mutations have been reported (<http://www.hgmd.org>). Nonsense and

frameshift mutations are common types of mutations resulting truncate the LAMP2 protein. The *LAMP2*p.Thr231Ilefs*11 variant is a novel frameshift mutation located on topological domain. This variant is not present in 1000Genomes browser (<http://browser.1000genomes.org/>), NHLBI ESP Exome Variant Server (<http://evs.gs.washington.edu/EVS/>) and genome Aggregation Database (gnomAD; <http://gnomad.broadinstitute.org/>). This variant is classified as pathogenic according to the guideline of the American College of Medical Genetics Laboratory Practice Committee Working Group [16].

In conclusion, we report a novel mutation in *LAMP2* gene and our study indicates that target NGS provides a rapid and highly sensitive screening method for the inherited cardiomyopathy.

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