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Case Report

Coexistence of a *CAV3* mutation and a *DMD* deletion in a family with complex muscular diseases

Takuya Hiraide [a,b,*], Tsutomu Ogata [a], Seiji Watanabe [c], Mitsuko Nakashima [b], Tokiko Fukuda [a], Hirotomo Saitsu [b]

[a] *Department of Pediatrics, Hamamatsu University School of Medicine, Hamamatsu, Japan*
[b] *Department of Biochemistry, Hamamatsu University School of Medicine, Hamamatsu, Japan*
[c] *Department of Pediatric Neurology, Shizuoka Children's Hospital, Shizuoka, Japan*

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Abstract

Whole-exome sequencing (WES) can comprehensively detect both pathogenic single nucleotide variants and copy number variants, enabling identification of a coexistence of two or more genetic etiologies. Here we report a family consisting of individuals with Becker muscular dystrophy and rippling muscle disease. The proband, a 12-year-old boy, was diagnosed with Becker muscular dystrophy with exon 45–55 *DMD* deletions at age 4. He had myalgia and muscle stiffness. Interestingly, percussion-induced muscle mounding (PIMM), which is a characteristic of rippling muscle disease, was also observed. The father also showed muscle stiffness, myalgia, fatigability, muscle rippling and PIMM. WES revealed a missense *CAV3* mutation (NM_033337.2:c.80G>A) in the proband, the father, the oldest sister and the grandmother, who had an elevated serum creatine kinase (CK) level. The c.80G>A mutation was considered pathogenic according to ACMG guidelines. The second older sister, the mother and the paternal grandfather did not have the *CAV3* mutation and had normal CK. Using two programs for copy number analysis with WES data, we successfully identified the *DMD* deletion in the proband, the older sister and the mother. We revealed the coexistence of the *CAV3* mutation and the *DMD* deletion in a family with complex muscular diseases and confirmed the usefulness of WES for elucidating such etiology.

Keywords: Becker muscular dystrophy; *CAV3*; *DMD*; Rippling muscle disease; Whole-exome sequencing

1. Introduction

Recent genomic approaches can find multiple genetic etiologies. Whole-exome sequencing (WES) is a well-developed method that can assist in diagnosing rare neuromuscular diseases, and it detects the coexistence of plural genetic etiologies. WES of a large cohort of subjects has revealed that two or more pathogenic variants can be found in 5% of patients as well as a single pathogenic variant [1].

Dystrophinopathies are X-linked recessive neuromuscular disorders characterized by progressive proximal muscular dystrophy, including cardiac muscles. Genetic defects involving *DMD* cause dystrophinopathies [2]. The patients develop severe Duchenne muscular dystrophy or the milder Becker muscular dystrophy (BMD) depending on whether the translational reading frame

* Corresponding author at: Department of Pediatrics, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-ku, Hamamatsu 431-3192, Japan.
E-mail address: thiraide@hama-med.ac.jp (T. Hiraide).

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is lost or maintained. For BMD patients, cardiac involvement is often the most important determinant of clinical status and outcome. Female carriers are usually asymptomatic with only elevated creatine kinase (CK), but some dystrophinopathy carriers have symptoms such as cardiomyopathy [2].

Rippling muscle disease (RMD) is a rare autosomal dominant disorder characterized by signs caused by unusually increased muscle excitability, such as muscle stiffness, stretching-induced involuntary muscle rippling, percussion-induced rapid contraction, percussion-induced muscle mounding (PIMM) and elevated CK. Heterozygous mutations in *CAV3* can cause RMD [3].

Dystrophinopathies and RMD are rare muscular disease. Here we report a family consisting of individuals with BMD and rippling muscle disease, which are caused by *DMD* deletion and *CAV3* mutation, respectively.

2. Case presentation

The family tree is shown in Fig. 1A, and clinical findings are summarized in Table 1. The proband (III-3) was a 12-year-old boy. Some of his family members had elevated serum CK. The oldest sister (III-1), the father (II-1) and the paternal grandmother (I-2) had hyperCKemia and the second older sister (III-2), the mother (II-2) and the paternal grandfather (I-1) had normal CK (Table 1). The proband (III-3) was born without asphyxia at 39 weeks 3 days of gestation, with a birth weight of 3334 g (0.54 standard deviation (SD)), a birth length of 52 cm (1.7 SD) and a birth head circumference of 33 cm (−0.21 SD). He began to walk unsupported at 1 year and 2 months of age, and began to speak meaningful words at 1 year of age. At age 4, his blood finding showed hyperCKemia. He was diagnosed with BMD due to a deletion of *DMD* exons 45–55 by Multiplex Ligation-dependent Probe Amplification (MLPA). At age 7, he developed muscle stiffness and myalgia when exercising in school and experienced PIMM. He was not able to run a long distance because of myalgia, and his symptoms were aggravated by tension and cold temperature. His muscle strength was normal and Gowers sign was negative. The tendon reflexes of his extremities were symmetrical, and no abnormal reflexes were found; however, his dorsiflexion of the ankle was limited and mild calf hypertrophy was observed (Fig. 2A). No percussion myotonia and no grip myotonia were observed. On blood test findings, his serum CK level was 4452 U/l (normal is <204 U/l). His electrocardiogram and echocardiography were normal. X-ray imaging showed no scoliosis of the spine. Skeletal muscle magnetic resonance imaging (MRI) of lower extremity revealed no abnormality (Fig. 1D–F).

The *DMD* deletion causing BMD in the proband did not explain an etiology of the elevated serum CK level in the father (II-1) and the grandmother (I-2). Therefore, we performed a detailed physical examination for the father (II-1) and investigated the genetic etiologies using WES.

The father (II-1) was 47 years old at the time of the study. At age 10, he was referred to a hospital for muscle stiffness, myalgia and fatigability during exercise. Even mild impacts caused severe muscle pain from myalgia. He experienced involuntary rippling in muscles most readily in the upper arm on extension of the upper extremities and the thigh on standing up. His symptoms were aggravated by cold temperature. At age 47, he was diagnosed with hyperCKemia during a health examination. His muscle strength was normal and Gowers sign was negative. He had no limitation of movement in any joint, but hyperreflexia in the biceps, triceps, brachioradial, patellar and Achilles tendon was observed. Mild calf hypertrophy was also observed (Fig. 2B). PIMM was seen in the extensor carpi radialis longus (Fig. 2C). Neither percussion myotonia nor grip myotonia was noted. We could not obtain informed consent to perform muscle biopsy. His serum CK level was 475 U/l. X-ray imaging of the spine revealed slight scoliosis of the lumber spine with a convexity to the right side. His electrocardiogram, spirometry, echocardiography, nerve conduction velocities and needle electromyography were normal. Muscle symptoms in other family members are described in Table 1.

3. Molecular analyses

This study was approved by the Institutional Review Board Committee at Hamamatsu University School of Medicine. After receiving written informed consent, genomic DNA extracted from peripheral blood samples from the proband and his family members were analyzed by WES. Data processing, variant calling, annotation and filtering were performed as previously described [4]. At least 96.9% of target coding sequences were covered by 20 reads. WES revealed a missense mutation in *CAV3* (NM_033337.2:c.80G>A, p.(Arg27Gln)) in the proband (III-3), the father (II-1), the oldest sister (III-1) and the grandmother (I-2). Sanger sequencing was performed to validate this pathogenic variant of *CAV3* (Fig. 1A). The c.80G>A mutation had been reported as an etiology of RMD and hyperCKemia [3] and registered as pathogenic in seven allele submissions in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/). The mutation is also absent in the gnomAD database (http://gnomad.broadinstitute.org/), and several programs have predicted it to be deleterious. Thus, the mutation is considered pathogenic according to ACMG Standards and Guidelines (PS1, PM2, PP1, PP2, PP3 and PP5).

Copy number variant (CNV) analysis using the WES data was also performed by the eXome Hidden Markov

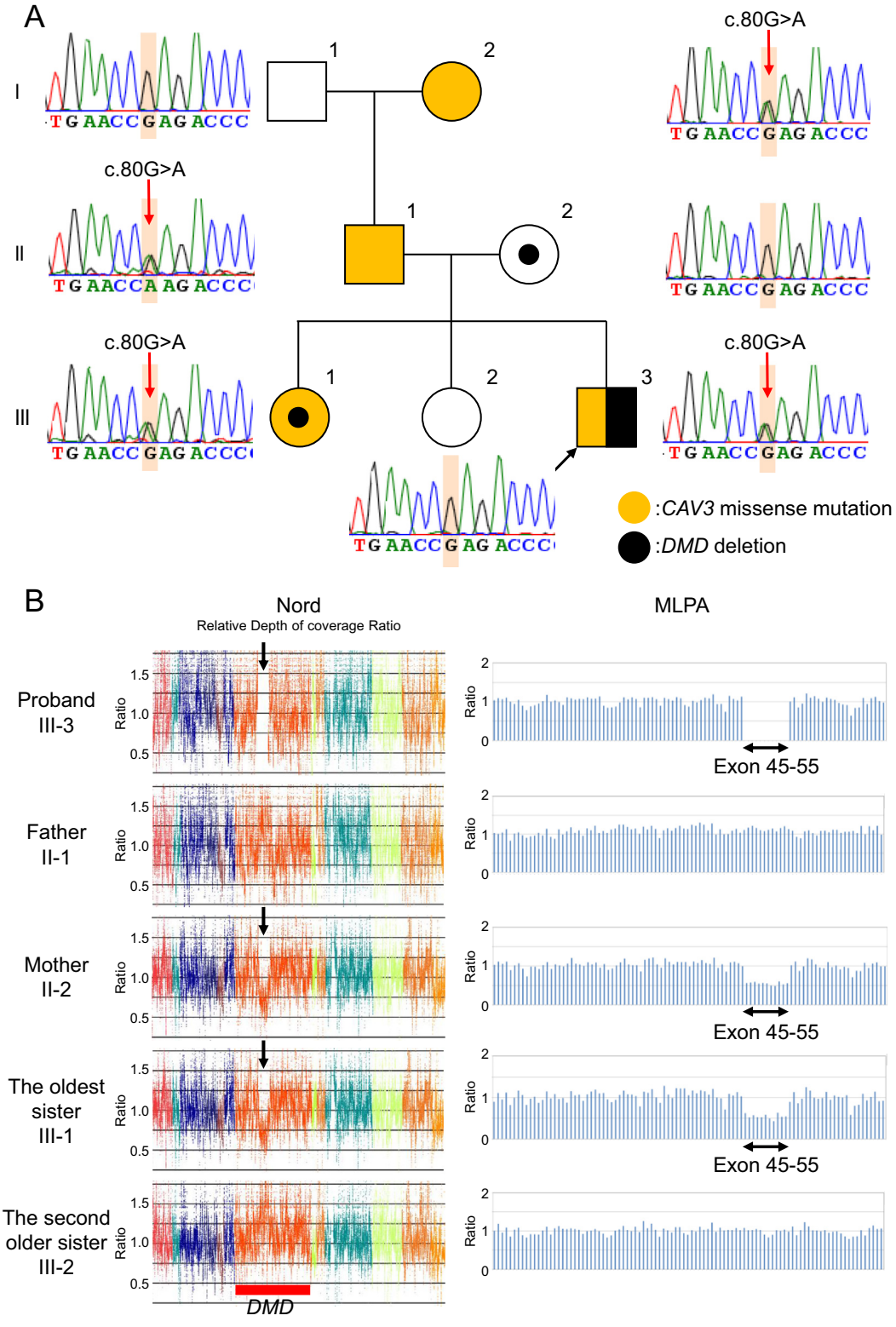

Fig. 1. (A) A family tree shows the individuals with the *CAV3* mutation in orange and the individuals with the *DMD* deletion in black. The black arrow indicates the proband. Sanger sequencing of the *CAV3* gene show a heterozygous missense mutation (NM_033337.2:c.80G>A) in the proband, the father, the oldest sister and the grandmother (red arrows). The proband and the oldest sister have both the *CAV3* mutation and the *DMD* deletion. (B) (Left) Relative depth of coverage ratio analysis using Nord's script in the family members. Coverage ratios for each target gene are indicated by different colors. The *DMD* is highlighted in red. The proband, the mother and the oldest sister have the *DMD* deletion (black arrow). (Right) MLPA analysis confirmed an exon 45–55 *DMD* deletion in the proband, the mother and the oldest sister. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Clinical findings of individuals of the family.

Individuals	Proband (III-3)	Father (II-1)	Mother (II-2)	The oldest sister (III-1)	The second older sister (III-2)	Grandfather (I-1)	Grandmother (I-2)
Genomic etiology							
CAV3 mutation	+	+	−	+	−	−	+
DMD deletion	+	−	+	+	−	−	−
Clinical characteristics							
Age	12 years	47 years	44 years	20 years	17 years	77 years	78 years
Serum CK level (55–204) (U/l)	4452	475	148	817	54	81	244
Fatigability	+	+	−	+	−	−	+
Myalgia	+	+	−	+	−	−	+
Stiffness	+	+	−	+	−	−	+
PIMM	+	+	−	−	−	−	+
PIRC	−	−	−	−	−	−	−
Rippling	−	+	−	−	−	−	−
Calf hypertrophy	+	+	−	+	NA	NA	NA

PIMM percussion-induced muscle mounding, PIRC stressed percussion-induced rapid contraction, NA not assessed or not available.

Model (XHMM) algorithm and by the relative depth of coverage ratio (Nord's script). XHMM detected a 340.87-kb deletion (GRch37/hg19; chrX:31645793–31986665, data not shown), including the *DMD* gene. Analysis by Nord's script showed the *DMD* deletion in the proband (III-3), the oldest sister (III-1) and the mother (II-2) (Fig. 1B, left). MLPA was performed to validate the predicted CNVs; the oldest sister (III-1) and the mother (II-2) had an exon 45–55 *DMD* deletion (Fig. 1B, right). This exon 45–55 *DMD* deletion was an in-frame deletion. These findings indicated that the oldest sister (III-1) and the mother (II-2) were carriers of BMD. The X-inactivation pattern was examined using the human androgen receptor assay, revealing that they did not present with skewed X chromosome inactivation (Supplemental Fig. 1).

4. Discussion

We reported a family with a very rare condition of having two pathological changes involving two genes that play important roles in maintaining muscle integrity. Careful evaluations of the family history and clinical findings of affected individuals suggested another etiology in this family, and we identified the *CAV3* mutation by WES. In the proband (III-3), the missense *CAV3* mutation and the *DMD* deletion were transmitted from his father (II-1) and his mother (II-2), respectively. The prevalence of dystrophinopathies is 1.38 per 10,000 male individuals [5]. *CAV3* mutations represent the 1% of unclassified limb girdle muscular dystrophy and various other muscle phenotypes of unknown etiology, including isolated elevated CK, RMD, distal myopathy, scapuloperoneal atrophy and unclassified hypertrophic cardiomyopathy [6]. This is the first report to identify very rare pathogenic variants of both *CAV3* and *DMD* in one family using WES.

Caveolin-3, encoded by *CAV3*, is a muscle-specific protein localized to the sarcolemma and is involved in the connection between the extracellular matrix and the cytoskeleton [7]. Caveolin-3 is associated with the biogenesis of the T-tubule system and it is suggested that defects of the T-tubule system cause fiber hyperexcitability [8]. Dystrophin, encoded by *DMD*, links the actin cytoskeleton to laminin in the extracellular matrix through the dystrophin associated protein complex. Dystrophin is postulated to play both structural and signaling roles in protecting muscle fibers from contraction-induced injury [2]. Caveolin-3 interacts with dystrophin and with the dystrophin associated glycoproteins [8]. The mother (II-2) is a carrier of the *DMD* deletion and her X chromosome inactivation pattern was random in blood leukocytes. Because she showed a normal CK level, it is postulated that the exon 45–55 in-frame *DMD* deletion may have mild adverse effects in multinucleated myocytes of carrier females. The oldest

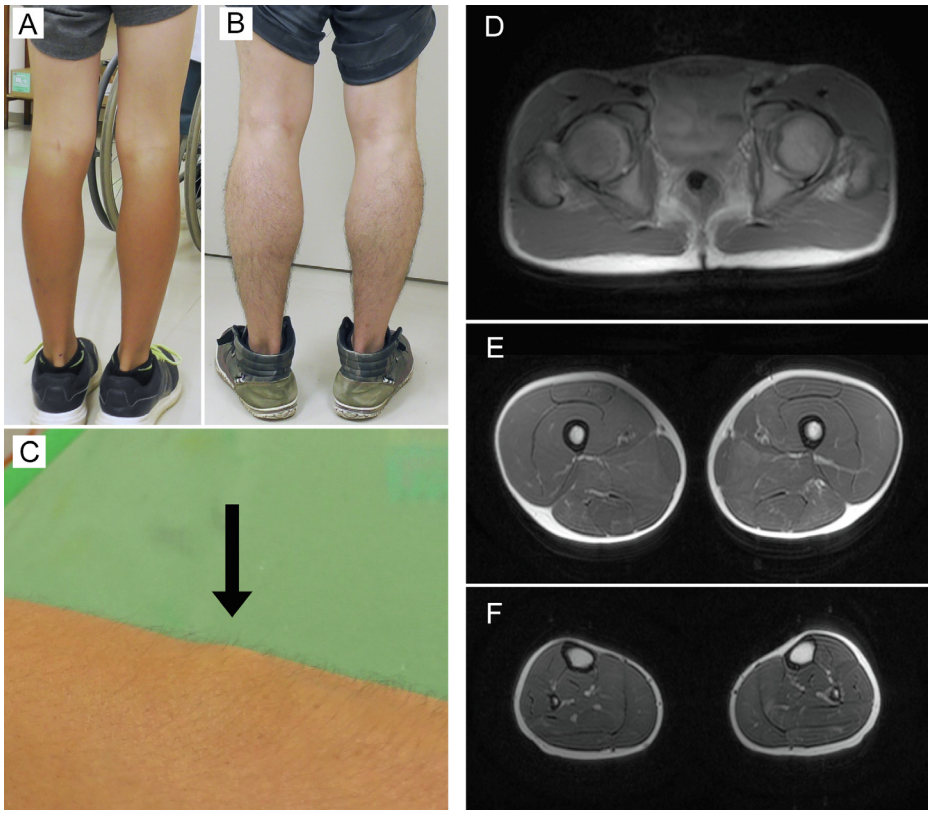

Fig. 2. (A) The proband (III-3), who presented with a *CAV3* mutation and a *DMD* deletion appearance, showed mild calf hypertrophy. (B) The father (II-1), who had a *CAV3* mutation, also had mild calf hypertrophy. (C) Percussion-induced muscle mounding was found in the extensor carpi radialis longus of the father (II-1) (arrow). (D–F) Skeletal muscle magnetic resonance imaging of pelvis (D), thigh (E) and calf (F) showed neither the atrophy nor the lipomatous degeneration.

sister (III-1) has both the *DMD* deletion and the *CAV3* mutation with a random X chromosome inactivation pattern. Interestingly, she showed a higher serum CK level than her father (II-1) and grandmother (I-2), both of whom also have the *CAV3* mutation. These findings suggest that coexistence of the *CAV3* mutation and the *DMD* deletion may work in conjunction to exaggerate muscle pathology, and this is believed to be the case in the proband (III-3).

Recently, it was reported that some patients with childhood-onset RMD showed distinct and characteristic skeletal muscle imaging findings [9]. However, the skeletal muscle MRI of our case showed no characteristic findings. The proband (III-3) had neither muscle weakness nor motor developmental delay unlike the previously reported cases [9]. It is possible that the characteristic findings of skeletal imaging may tend not to be discernible when RMD patients have no muscle weakness.

Using WES, we detected both pathogenic single nucleotide variants and CNV. WES has been reported as being useful in analysis for all of point mutations, small indels, and CNVs [10]. Furthermore, because next-generation sequencing is widely used, multiple etiologies have begun to be found. However, WES can reveal unexpected gene alternations as secondary findings. If pathogenic mutations are detected prior to the onset of symptoms, managing of secondary findings would be controversial. When treatment of the disease caused by the identified mutation is extremely effective, it may be necessary to return results from WES. In any case, clinicians should explain possibility of secondary findings to individuals and family members before undergoing WES.

In conclusion, we identified a rare coexistence of a *CAV3* mutation and a *DMD* deletion by an exome analysis and CNV analysis tool in a family having unknown etiology of hyperCKemia. This shows that careful evaluation of the motor function and the cardiac function in affected family members is necessary.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.braindev.2019.01.005.

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