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CLINICAL REPORT

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Two novel mutations in *TTN* of a patient with congenital myopathy: A case report

Joon Young Jang[1] | **Yulhyun Park**[1] | **Dae-Hyun Jang**[2] | **Ja-Hyun Jang**[3] | **Ju Seok Ryu**[1]

[1]Department of Rehabilitation Medicine, Seoul National University Bundang Hospital, Seoul National University College of Medicine, Seongnam, Korea

[2]Department of Rehabilitation Medicine, College of Medicine, The Catholic University of Korea, Seoul, Korea

[3]Green Cross Genome, Yongin, Korea

Correspondence
Ju Seok Ryu, Department of Rehabilitation Medicine, Seoul National University Bundang Hospital, Seoul National University College of Medicine, 82 Gumi-ro 173 Beon-gil, Bundang-gu, Seongnam-si, Gyeonggi-do 13,620, Republic of Korea.
Email: jseok337@snu.ac.kr

Dae-Hyun Jang, Department of Rehabilitation Medicine, Incheon St. Mary's Hospital, The Catholic University of Korea, 56, Dongsu-ro, Bupyeong-gu, Incheon, 21,431, Republic of Korea.
Email: dhjangmd@naver.com

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Abstract

Background: Early-onset myopathies show a wide spectrum of phenotypes and are composed of various types of inherited neuromuscular diseases, making it difficult to diagnose. *TTN* mutation-related myopathy is a known cause of early-onset myopathy. Since a next-generation sequencing (NGS) has enabled sequencing of the vast amount of DNA, *TTN*, which is the longest coding sequence of any human gene, mutations can now be revealed. We report a 10-year-old female with severe congenital muscular weakness and delayed motor development since birth.

Methods: Next-generation sequencing as well as electromyography and muscle biopsy were performed.

Results: To date, she is incapable of walking alone. Her younger sister had similar but more severe symptoms with respiratory failure. In electromyography, short-duration, small-amplitude motor unit action potential, and early recruitment patterns were observed in the involved proximal muscles, suggesting myopathy. Muscle histopathology showed a specific atrophy of increased fiber size variability, frequent nuclear internalization, as well as degeneration and regeneration of fibers with type I fiber predominance, consistent with the findings of a previous report about congenital titinopathy. A NGS study revealed two different possible pathogenic variants in *TTN*: (a) canonical splicing mutation in the intron 105 (c. 29963-1G>C) and (b) frameshift and truncating mutation in the exon 339 (c.92812dup/p.Arg30938LysfsTer15). All variants were confirmed by conventional Sanger sequencing.

Conclusion: We propose that unbiased genomic sequencing can be helpful in screening patients with early-onset myopathy.

KEYWORDS
congenital myopathies, human, next-generation sequencing, *TTN*

Ju Seok Ryu and Dae-Hyun Jang contributed equally to this paper and should therefore be regarded as equivalent corresponding authors.

1 | INTRODUCTION

TTN (OMIM# 188,840) provides instructions for making a large protein, titin, that provides structural support, flexibility, and stability within the sarcomere (Bang et al., 2001; Kruger & Kotter, 2016). Since titin plays a crucial role in the sarcomere, mutations in *TTN* may cause a wide spectrum of striated muscle diseases, including tibial muscular dystrophy, hereditary myopathy with early respiratory failure, and limb-girdle muscular dystrophy type 2J (LGMD2J) (Bang et al., 2001; Hackman et al., 2002; Pfeffer et al., 2014).

The next-generation sequencing technique has enabled the elucidation of several genetic diseases associated with neuromuscular disease and is being rapidly implemented into routine clinical practice (Helman, Bonkowsky, & Vanderver, 2016).

In this study, we describe a patient with two novel heterozygous *TTN* mutations presenting severe muscular weakness.

1.1 | Ethical compliance

We obtained written informed consent from the patient. This case report was approved by the Institutional Review Board of the Seoul National University Bundang Hospital, and it was conducted in accordance with the tenets of the Declaration of Helsinki (IRB number: B-1705-399-301).

2 | CASE REPORTS

A 10-year-old female patient visited the outpatient clinic of the Department of Rehabilitation Medicine for further evaluation of severe motor weakness, hypotonia, and poor sucking ability. There were no significant abnormal findings during the pre- and perinatal periods; however, motor development was delayed since birth. To date, she was never able to walk alone. On manual muscle test, she got a grade of 2 for proximal muscles and a grade of 3 for distal muscles in all upper and lower extremities. On the range of motion (ROM) test, the knee flexion contracture on the right side was 30°. No upper motor neuron sign was observed. Deep tendon reflexes were absent. She also showed suspicious tongue fasciculation and funnel chest. Cognitive function was normal and had no facial abnormalities. There was no history of delayed development, weakness or other neuromuscular abnormalities among the first-degree relatives of her father (one older sister and two older brothers) and mother (two younger sisters), except her younger sister (Figure 1a). Her younger sister had similar symptoms, but with greater severity and respiratory failure. Creatine kinase (CK) levels were within normal limits. On X-ray studies, thoracolumbar scoliosis (the Cobb angle was 20°) and bilateral coxa valga (the caput-collum-diaphyseal (CCD) angles were 142°, 146°, right, and left side, respectively) were observed. Brain MRI showed no specific abnormality.

Nerve conduction studies showed normal findings (Table 1). On electromyography, short-duration, small-amplitude motor unit action potential, and early recruitment patterns were observed in the involved proximal muscles, suggesting myopathy (Table 2).

The *SMN* study showed no abnormal findings. Genetic testing was performed using a targeted gene sequencing panel, analyzing 410 genes associated with genetic

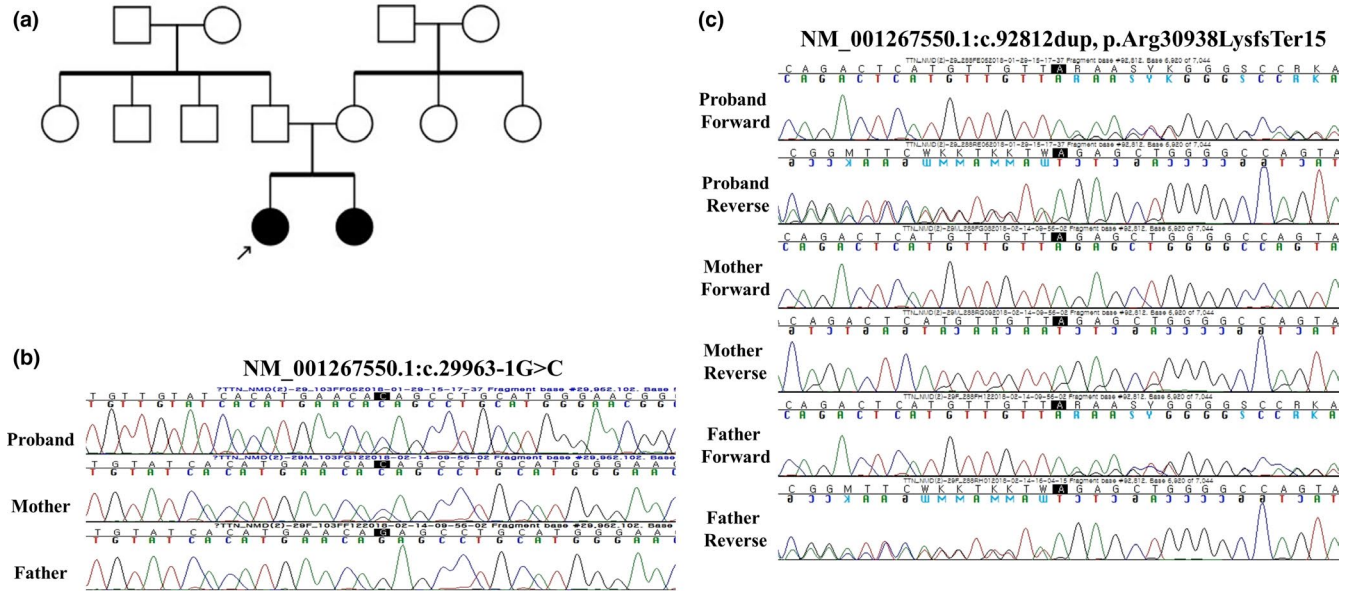

FIGURE 1 (a) Pedigree of the family. Two different possible pathogenic variants in *TTN*, which were related with muscular dystrophy: (b) canonical splicing mutation in the intron 105 (c. 29963-1G>C) from the mother; and (c) frameshift and truncating mutation in the exon 339 (c.92812dup/p.Arg30938LysfsTer15) from the father

TABLE 1 Nerve conduction study and summary

Nerve		Stimulation Site	Recording Site	Latency (msec)	Amplitude Sensory = uV Motor = mV	Conduction velocity (m/s)
Motor						
Median	Right	Wrist	APB	2.03 (<4.2)	7.6 (>5.0)	
		Elbow	APB	4.79	6.6 (>5.0)	68.8 (>50.0)
Ulnar	Right	Wrist	ADM	1.88 (<4.2)	8.2 (>5.0)	
		Below elbow	ADM	4.74	7.8 (>5.0)	73.3 (>50.0)
Peroneal	Right	Ankle	EDB	2.08 (<6.0)	2.7 (>2.0)	
		Fibular head	EDB	5.99	2.4 (>2.0)	58.9 (>50.0)
Tibial	Right	Ankle	AH	2.19 (<5.0)	13.3 (>5.0)	
		Popliteal fossa	AH	7.03	10.5 (>5.0)	55.7 (>50.0)
Sensory						
Median	Right	Wrist	II digit	1.88 (<3.2)	163.1 (>20.0)	74.5 (>50.0)
Ulnar	Right	Wrist	V digit	1.82 (<3.1)	166.0 (>10.0)	76.9 (>50.0)
Peroneal	Right	Lateral leg	Ankle	1.82 (<3.1)	50.9 (> 5.0)	76.9 (>50.0)
Sural	Right	Posterior leg	Foot	1.82 (<3.1)	31.6 (> 10.0)	76.9 (>50.0)

Note: All sensory nerve responses were antidromic. Limb temperature was maintained ≥32°C.
Abbreviations: ADM, Abductor digitorum minimi; AH, abductor halluces; APB, Abductor pollicis brevis; EDB, extensor digitorium brevis; Normal, normal values.

neuromuscular diseases. Genomic DNA was extracted from the peripheral blood of the patient. Library preparation and target enrichment were performed using the hybridization capture method. Custom oligo design and synthesis were done by Agilent (USA). Massively parallel sequencing was performed using 2 × 150 bp in the paired end mode of NextSeq platform (Illumina, San Diego, CA). Sequence reads were aligned with the Burrow–Wheeler Aligner (version 0.7.12, MEM algorithm, MEM algorithm). After removing the duplicated reads with the Picard, local realignment and recalibration were performed using the Genome Analysis Tool Kit (GATK, version 3.5). Variants were annotated by Variant Effect Predictor and dbNSFP. Common variants with a minor allele frequency ≥ 1% were filtered out using public databases (i.e., 1,000 Genomes Project, Exome Variant Server, Exome Aggregation Consortium, and The Genome Aggregation Database). The average coverage depth was 182×, and 96.6% of the target bases were covered by more than 10 × sequence reads.

We found two different possible pathogenic variants in *TTN* (NM_001267550.1), which were related to muscular dystrophy: canonical splicing mutation in the intron 105 (c. 29963-1G > C) from the mother; and frameshift and truncating mutation in the exon 339 (c.92812dup/p. Arg30938LysfsTer15) from the father. All variants were confirmed by Sanger sequencing (Figure 1b,c). The patient's parents were identified as heterozygous carriers for each variation occurring in trans. These novel variations have not been reported in the control databases, such as the 1,000 Genomes Project, Exome Aggregation Consortium, Exome Variant Server, dbVar, and the dbSNP Database.

For confirmation, muscle biopsy samples were taken from the vastus lateralis muscle. Immunohistochemical and histologic studies, including the electron microscopic exam,

TABLE 2 Electromyographic analysis and summary

Side	Muscle	Insertion Activity	Fibs	PSW	Amplitude	Duration	Polyphasic Potentials	Recruitment
Right	Vastus medialis	**Decreased**	None	None	Normal	**Decreased**	**Increased**	**Early**
Right	Tibialis anterior	Normal	None	None	Normal	Normal	**Increased**	**Early**
Right	Biceps brachii	Normal	None	None	Normal	**Decreased**	**Increased**	**Early**
Right	Flexor carpi radialis	Normal	None	None	Normal	**Decreased**	**Increased**	**Early**

Note: Bold—abnormal findings.
Abbreviations: Fibs, fibillation potentials; PSW, positive sharp waves

were performed. Histochemical evidence showed a specific atrophy of increased fiber size variability, frequent nuclear internalization, as well as the degeneration and regeneration of fibers with type I fiber predominance on muscle biopsy. On electron microscopy, we did not observe any nemaline body, centronuclear core, or multiminicore.

3 | DISCUSSION

The evidence presented here suggests that these frameshift and truncating mutations as well as canonical splicing mutations are the "pathogenic" variants, based on the American College of Medical Genetics and Genomics guidelines regarding the interpretation of sequence variations (PSV1 + PM2 + PP4) (Richards et al., 2015). We also think that these two novel mutations may be the causative of the phenotypes in our patient.

Since the NGS technique enabled the sequencing of *TTN*, which is the longest coding sequence of any human gene, there is an increasing number of rising cases regarding the genetic diseases associated with *TTN*. A recent article published in JAMA neurology (Savarese et al., 2018) showed that a patient may be susceptible to the diagnosis of titinopathy, since these mutations are biallelic protein truncating variants.

Similar to previous studies that reported various phenotypes of *TTN*-related myopathy (Harris et al., 2017; Oates et al., 2018; Savarese et al., 2018), our patient represented axial involvements, including neck weakness, scoliosis, funnel chest, muscle weakness (proximal > distal), limb contracture, and respiratory difficulties. The muscle biopsy result of the patient is increased fiber size variation and frequent internalized nuclei. These are two of the three main patterns that are found in the previous report on congenital titinopathy. The result of the fiber type analysis was degeneration and regeneration of fibers with type I fiber predominance, which is consistent with the result of the report (Oates et al., 2018). Compared with patients reported in previous studies (Udd, Kaarianen, & Somer, 1991; Udd, Rapola, Nokelainen, Arikawa, & Somer, 1992), our patient experienced extremely early onset. This might be due to "two-truncation" mutations in *TTN*, as suggested by a previous study (Harris et al., 2017).

In conclusion, early-onset myopathy can be caused by various types of diseases, including genetic diseases. *TTN* mutation causes a wide spectrum of genetic diseases that present skeletal and/or cardiac myopathies; primary pathogenic gene mutation may determine these phenotypic variances that result in functional/structural damage in the sarcomere.

There are two limitations to this study. First, we were unable to examine the gene of the patient's sister. We were unable to perform blood tests because the patient's sister was on a ventilator due to severe respiratory failure and resided far from the hospital. Second, we were unable to further characterize our canonical splice site variant. As splice site mutation can result in the production of a near-normal sized protein product (Oates et al., 2018), it is important to fully characterize our variant and see whether the splicing effect really causes truncation for a better understanding of the molecular causes of the disease. When we searched the HGMD professional database (2019.1), there was 18 splicing-related mutations, out of a total of 148 mutations, which were mostly truncating variants. Hence, together with our patient's symptoms, the result of examinations, and previously reported on HGMD professional database, we can highly suggest that our splicing variant is disease-causing mutation.

Since a massive evolution in genetic analyses was achieved, which enabled a comprehensive evaluation of titinopathy, we propose that unbiased genomic sequencing can be helpful in screening patients with early-onset myopathy.

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CONFLICT OF INTEREST

The authors have no conflict of interest to disclose and there was no financial support.

ORCID

Dae-Hyun Jang https://orcid.org/0000-0001-8293-084X
Ju Seok Ryu https://orcid.org/0000-0003-3299-3038

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