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CASE REPORT Open Access

Heterozygous missense variant in the *TTN* gene causing Tibial muscular dystrophy

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Abstract

Background: Tibial muscular dystrophy (TMD), tardive, is a dominantly inherited mild degenerative disorder of anterior tibial muscles. Mutations of *Titin (TTN)* have been reported in patients with different phenotypes such as skeletal muscular abnormalities or complex overlapping disorders of muscles. *Titin* (*TTN*) is a large 363 exon gene that encodes an abundant protein (the longest polypeptide known in nature) expressed in the heart and skeletal muscles.

Methods: DNA from peripheral blood sample was extracted, whole exome sequencing (WES) was performed, and a neuromuscular disorders related gene-filtering strategy was used to analyse the disease-causing mutations. Further, sanger sequencing was applied to confirm the variant.

Results: A novel missense variant (c.41529G > C;p.Arg13843Ser) of *TTN* gene was identified in a patient with lower limb weakness, occasional tongue fasciculation and mild scoliosis. This variant leads to a substitution of arginine with serine, causing structural changes in titin protein that is responsible for the TMD disease.

Conclusion: The novel variant detected has widened the genetic spectrum of *TTN*-associated diseases, further functional studies will aid in establishing the clinical diagnosis.

Keyword: Tibial muscular dystrophy (TMD), *TTN*, Whole exome sequencing, Variant, Sanger sequencing

Background

Tibial muscular dystrophy (TMD) is a late-onset distal myopathy with an autosomal dominant inheritance pattern. It was first described in a Finnish patient affecting at least one in 10,000 people [1, 2]. In patients with the mildest form of TMD, symptoms may go unnoticed, are usually confined to the lower leg in particular, the tibialis anterior muscle, and appear between the ages of 35 to 45 years. The strength of the muscles just below the ankle can be affected by muscle weakness in the ankle [2, 3].

With the progressive onset, weakness and atrophy of the long toe extensors make it difficult to lift the foot while walking, causing a condition called foot drop. This can cause clumsiness and difficulty in walking. But despite the difficulties, most patients retain the ability to walk [4, 5]. In rare cases, this condition can weaken the arm muscles, but cardiomyopathy and involvement of the facial muscles have not been diagnosed in patients with TMD [2, 6].

TMD is caused by mutations in the *TTN* gene that carries the instructions for making a protein called titin [7]. The *TTN* gene is on chromosome 2 (2q31); the entire coding region consists of 363 exons encoding 38,138 amino acid residues (4200 kD) [8]. The terminal ends of titin are embedded in two specific sarcomeric regions; the N-terminus within the Z-disc, and the C-terminus within the M-line. Mutation in extreme C terminus of *TTN*, situated at the end of M-band of the *TTN*, results

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in TMD. One of the best characterized functions of titin is that of a scaffold protein aiding myofibrillar assembly during myogenesis, and it is responsible for muscle stretching [9]. However, it is also the backbone to keep the contractile elements of the sarcomere in place [10, 11]. An important role in the myofibrillar signalling pathway has also been demonstrated, and Titin appears to integrate or coordinate multiple signalling pathways involved in gene activation and/or protein folding, quality control and degradation [12–14].

Mutations in the *TTN* gene lead to the production of a defective titin protein, the structure and function of which are altered. This defective protein titin impairs the function of sarcomeres and normal muscle contraction. The severity of the symptoms of TMD is determined by the type of *TTN* mutation and varies from patient to patient. The effects of mutations in the *TTN* gene in the muscles of the lower leg are yet to be ascertained [15].

Many additional *TTN*-related muscular phenotypes are emerging as a consequence of next-generation sequencing (NGS) screening in patients with myopathy [16]. Whole exome sequencing (WES) is rapidly being implemented in genetic diagnostic practice for patients with neuromuscular diseases [17–19]. *TTN* gene is huge in size, makes it difficult to sequence the entirely on a routine basis in diagnostic laboratories. Therefore, before the introduction of NGS technology, only a limited number of *TTN* mutations were identified. WES has enabled the rapid identification of new *TTN* variants [20]. However, NGS screenings reveal many rare titin variants but their clinical interpretation is a challenge [16, 21, 22].

The present study detects the novel *TTN* gene variant in patient with neuromuscular disorders and provides a path for further functional studies to establish clinical diagnosis.

Case presentation

A 20-year-old male with no bilateral facial weakness/ophthalmoplegia was presented with 2-year long history of walking difficulties. While walking he has to drag the feet and upon examination was notable for severe lower limb weakness and occasional tongue fasciculation. Further examination also showed moderate weakness of abductor digiti minimi (ADM) and mild scoliosis. In clinical investigation, magnetic resonance imaging (MRI) was conducted for Cervico Dorsal Spine and showed no abnormality, cervical spine was normal in curvature and alignment, and intervertebral discs were also normal in height. While in blood test, marker for the detection of skeletal muscle disease, CK-NAC [N-acetyl-cystein-(NAC)-activated creatinkinase (CK)] was elevated to 672 U/L and uric acid was found to be in normal range along with non-reactive HBsAg (Hepatitis B surface antigen). Nerve conduction study (NCS) was normal. Needle electromyography (EMG) showed denervation active patterns in First Dorsal Interosseous (FDI) muscles. To further investigate the diagnosis, Whole Exome Sequencing was performed which revealed a novel heterozygous missense variant (c.41529G > C;p.Arg13843Ser) in *TTN* gene. DNA was extracted using Qiagen DNA mini kit, as per the manufacturer's instructions. The quality of extracted genomic DNA (gDNA) was measured with a NanoDrop2000 spectrophotometer and then with a Qubit 3.0 fluorometer for more accurate DNA quantification using the Qubit dsDNA High Sensitivity (HS) Assay Kit. Approximately 100 ng of genomic DNA was taken for exome libraries construction employing the Ion Ampliseq Exome RDY panel (Thermo Fisher Scientific) according to the manufacturer's protocol.

Sequencing was done using Hi-Q chemistry on Ion Proton platform (Thermo Fisher Scientific). Sequences were aligned against the reference genome (GRCh37/hg19) in Torrent Suite v.5.12.0 and Torrent Suite Variant Caller v.5.2.1 software (Thermo Fisher Scientific), with default parameters followed by annotation of VCF file using Ion Reporter v5.18 (Thermo Fisher). A total of 38,767 variants composed of 54% synonymous, 43% missense and 2% frameshift/indels were found in the WES data. For WES data filtering procedures, first phase consisted of benign and synonymous variant filtering, and the second phase was based on variant impact (nonsynonymous, truncating), allele frequency (< 0.1%) and pathogenicity prediction tools for missense variants (score > 3). Since, there were still many candidate variants and genes, we performed a second prioritization step based on manual regulation of biological function and filtered out variants of genes not relevant to the patient's phenotype. WES results indicated a novel heterozygous missense variant (c.41529G > C;p.Arg13843Ser) in *TTN* gene responsible for Tibial muscular dystrophy. Identified variant was located in GC region and variant coverage and alignment at position chr2:179,484,592 was viewed using the Integrative Genomics Viewer (IGV, http://www.broadinstitute.org/igv/) (Fig. 1B). Confirmation of the identified variant was done by Sanger sequencing using the BigDye™ Terminator v3.1 Cycle Sequencing kit and loaded on an ABI 3500Dx automated Genetic Analyzer (Applied Biosystems, Thermo Fisher Scientific). Primer sequences for the pathogenic variant in the *TTN* gene (NM_001256850.1) were designed using Primer 3.0 online as follow: Forward 5′-ATGCTTGCGGGGTAG AAGAC-3′ and Reverse 5′- CCTGGTCACGGGGCT TAAT-3′. Sanger sequencing analysis confirmed that the proband carried the variant in heterozygous state (Fig. 1C). The pathogenicity of detected variant was evaluated using different online bioinformatics tools; SIFT

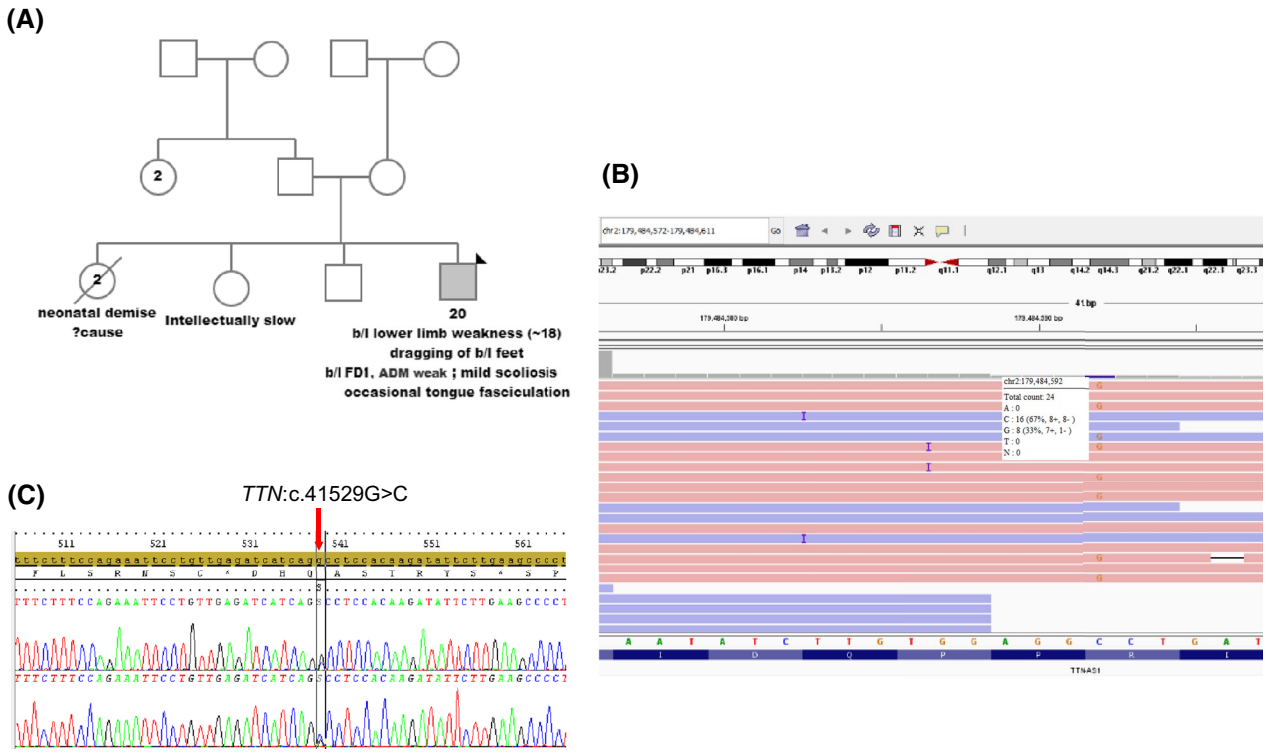

Fig. 1 **A** Pedigree of the probands' family. **B** IGV plot showing the mutation region in WES data in the proband. Track comprises two parts: a histogram of the read depth and the reads as aligned to the reference sequence. Reads are colored according to the aligned strand (red = forward strand; blue = reverse strand). **C** Sanger sequence chromatogram showing a novel heterozygous missense variant in exon 200 of the *TTN* gene (c.41529G > C;p.Arg13843Ser) associated with tibial muscular dystrophy

(https://sift.bii.a-star.edu.sg/), Mutation Taster (http://www.mutationtaster.org), PROVEAN (http://provean.jcvi.org/index.php), and FATHMM-XF (http://fathmm.biocompute.org.uk/fathmm-xf/) which predicted this variant to be damaging (Table 1). Finally, for the interpretation of variant, American College of Medical Genetics and Genomics (ACMG) 2015 guidelines were followed [23].

Discussion

TTN performs several critical functions in all skeletal muscle cells that are well adapted to its role as an architectural protein and provide specific binding to a variety of essential proteins [24]. *TTN* had previously been linked to cardiovascular syndromes until Hackman and his colleagues identified it as a cause of tibial muscular dystrophy (TMD) (OMIM #600334) in a Finnish population in the year 2002 [25]. In addition to Finnish population, disease-causing *TTN* variants in TMD patients were also reported in other European families, enlisted in Table 2. TMD is a dominant, distal myopathy which typically presents in adulthood. Weakness usually affects the anterior tibial muscle and does not cause cardiomyopathy. Hackman and co-workers were the first to report *TTN* as a cause of skeletal muscle disease and demonstrated that a variant of Mex6 (the last exon of TTN) causes a functional defect in the titin M line and results in a predominantly inherited TMD phenotype.

Several research groups have used next generation sequencing to assess the role of *TTN* variants in skeletal

Table 1 WES analysis of identified the *TTN* gene variant in the proband

Locus	Gene	Exon	Protein	Coding	Mutation Taster	SIFT	PROVEAN	FATHMM-XF
chr2:179484592	*TTN*	200	p.Arg13843Ser	c.41529G > C	D	D	D	D

D, damaging or deleterious

Table 2 Reported variants in *TTN* gene causing TMD in European families

Patient family	Mutation	References
Finnish family	c.102857_102867delinsTGAAAGAAAAA (p.Glu34286_Trp34289delinsValLysGluLys)	Hackman et al. [25]
Finnish family	c.102944T > C (p.Leu34315Pro)	Hackman et al. [25]
French family	c.107867T > C (p.Leu35956Pro)	Hackman et al. [25], de Seze et al. [26]
French family	c.107890C > T (p.Gln35964*)	Hackman et al. [27]
Albacete family	c.107889delA (p.Lys35963Asnfs*9)	Hackman et al. [27]
Barcelona family	c.107889delA (p.Lys35963Asnfs*9)	Hackman et al. [27]
Belgian family	c.107840T > A (p.Ile35947Asn)	van den Bergh et al. [4]
Belgian family	c.102917T > G (p.Ile34306Ser)	van den Bergh et al. [4]
Italian family	c.107837A > C (p.His35946Pro)	Pollazzon et al. [5]

disease. Savarese and his group attempted to distinguish positive *TTN* variants from pathogenic variants by iteratively sequencing patients with skeletal myopathy ($n=504$) with uncharacterized disease [28]. The classification of *TTN* variants identified by WES is problematic because of the large number of variants of this gene, both in size and predominance of heterozygous variants, which are reduced to 2% of the normal population [29–31]. Using WES, we detected heterozygous missense variant (c.41529G > C;p.Arg13843Ser) which was confirmed by Sanger DNA sequencing in exon 200 of *TTN* gene, associated with TMD phenotypes. In order to assess the degree of pathogenicity, functionality and stability of the protein, the identified variant was subjected to various in silico functional prediction algorithms based on criteria such as the location of the variant on the genome, sequence homology, degree of conservation and physicochemical properties and structure. SIFT, MutationTaster, PROVEAN and FATHMM-XF protein function prediction softwares were used, and all agreed on the pathogenicity of the variant. The results from these tools classified the variant as deleterious and disease-causing, and boosted the variant pathogenicity level on the protein structure.

However, definitive evidence of pathogenicity for missense variants can only be established by functional study, segregation studies in very large families and/or by identifying unrelated patients or families with the same mutation. The interpretation of the missense variant of *TTN* could also benefit from establishing a clinical and research consortium able to incorporate a group of patients into a larger cohort [32].

Diagnostic laboratories today are making widespread use of next-generation whole sequencing, which is increasing the number of *TTN* variants (particularly those of uncertain significance) enrolled in clinical testing. This then presents a challenge for clinicians who need to assess the importance of *TTN* variants as part of their diagnostic assessment. Furthermore, the high rates of *TTN* variants in the general population limits the understanding of pathogenicity [33]. It is estimated that at least three rare, non-synonymous *TTN* variants are identified in any one individual, which is certainly related to the sheer size of the gene [16]. Often these rare/novel variants, as well as missense variants are classified as variants of uncertain significance (VUS). Uncertainties associated with VUS in TTN, especially those associated with skeletal myopathy, inevitably complicate the diagnostic work-up, including genetic counselling and clinical management.

Previous studies have confirmed the association of *TTN* variants with several life-threatening neuromuscular and/or cardiovascular disease, though both interpretation and clinical utility of *TTN* variants are often challenging in this setting [16, 31, 34, 35]. Although *in-silico* predictions of the effects of any missense variant are frequently unreliable, functional validation of missense changes in *TTN* presents unique challenges as its large size prevents cloning and expression of full-length protein in *in-vitro* systems [16].

However, despite recent reports reducing overall population estimates of *TTN* mutations, historical awareness of the prevalence of *TTN* mutations remains unexcavated which further exacerbate the problem of diagnostic interpretation.

Conclusions

WES has dramatically expanded the spectrum of skeletal muscle disorders associated with *TTN* causative mutation. Our study sensitizes the neurologists and geneticists on the potential role of *TTN* gene and titinopathies thereby, aiding in better understanding and more consistent interpretation of titin mutations.

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Authors' contributions
DP and AT conceived and designed the experiments. DP and KGS processed the data, conceptualized and conceived the analytical methods. DP drafted the manuscript. AT and VL supervised the study and was in charge of overall professional scientific direction and planning. SM counselled the patient. BP and AJ performed the wet laboratory work. All authors read and approved the final manuscript.

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Availability of data and materials
The data used to support the findings of the present study are included within the article. Data will be made available on demand.

Declarations

Ethics approval and consent to participate
The study design and protocol were conducted in accordance with the guidelines of the ACMG, and was approved by Ethical Review Committee (ERC) of Dr. Lal Pathlabs.

Consent to publication
Written informed consent has been taken to publish the findings from the patient considered in this study.

Competing interests
The authors declare no conflict of interest.

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References

1. Udd B, Kaarianen H, Somer H (1991) Muscular dystrophy with separate clinical phenotypes in a large family. Muscle Nerve 14(11):1050–1058
2. Udd B, Partanen J, Halonen P, Falck B, Hakamies L, Heikkila H et al (1993) Tibial muscular dystrophy. Late adult-onset distal myopathy in 66 Finnish patients. Arch Neurol 50(6):604–608
3. Haravuori H, Vihola A, Straub V, Auranen M, Richard I, Marchand S et al (2001) Secondary calpain3 deficiency in 2q-linked muscular dystrophy: titin is the candidate gene. Neurology 56(7):869–877
4. van den Bergh PY, Bouquiaux O, Verellen C, Marchand S, Richard I, Hackman P et al (2003) Tibial muscular dystrophy in a Belgian family. Ann Neurol 54(2):248–251
5. Pollazzon M, Suominen T, Penttila S, Malandrini A, Carluccio MA, Mondelli M et al (2010) The first Italian family with tibial muscular dystrophy caused by a novel titin mutation. J Neurol 257(4):575–579
6. Udd B, Rapola J, Nokelainen P, Arikawa E, Somer H (1992) Nonvacuolar myopathy in a large family with both late adult onset distal myopathy and severe proximal muscular dystrophy. J Neurol Sci 113(2):214–221
7. Haravuori H, Makela-Bengs P, Udd B, Partanen J, Pulkkinen L, Somer H et al (1998) Assignment of the tibial muscular dystrophy locus to chromosome 2q31. Am J Hum Genet 62(3):620–626
8. Bang ML, Centner T, Fornoff F, Geach AJ, Gotthardt M, McNabb M et al (2001) The complete gene sequence of titin, expression of an unusual approximately 700-kDa titin isoform, and its interaction with obscurin identify a novel Z-line to I-band linking system. Circ Res 89(11):1065–1072
9. Labeit S, Gautel M, Lakey A, Trinick J (1992) Towards a molecular understanding of titin. EMBO J 11(5):1711–1716
10. Linke WA, Stockmeier MR, Ivemeyer M, Hosser H, Mundel P (1998) Characterizing titin's I-band Ig domain region as an entropic spring. J Cell Sci 111(Pt 11):1567–1574
11. Linke WA, Kulke M, Li H, Fujita-Becker S, Neagoe C, Manstein DJ et al (2002) PEVK domain of titin: an entropic spring with actin-binding properties. J Struct Biol 137(1–2):194–205
12. Kruger M, Linke WA (2009) Titin-based mechanical signalling in normal and failing myocardium. J Mol Cell Cardiol 46(4):490–498
13. Kruger M, Linke WA (2011) The giant protein titin: a regulatory node that integrates myocyte signaling pathways. J Biol Chem 286(12):9905–9912
14. Kravchenko IV, Furalyov VA, Chatziefthimiou S, Wilmanns M, Popov VO (2015) Induction of insulin-like growth factor 1 splice forms by subfragments of myofibrillar proteins. Mol Cell Endocrinol 399:69–77
15. Chauveau C, Rowell J, Ferreiro A (2014) A rising titan: TTN review and mutation update. Hum Mutat 35(9):1046–1059
16. Savarese M, Sarparanta J, Vihola A, Udd B, Hackman P (2016) Increasing role of titin mutations in neuromuscular disorders. J Neuromuscul Dis 3(3):293–308
17. Thompson R, Straub V (2016) Limb-girdle muscular dystrophies - international collaborations for translational research. Nat Rev Neurol 12(5):294–309
18. Nigro V, Savarese M (2016) Next-generation sequencing approaches for the diagnosis of skeletal muscle disorders. Curr Opin Neurol 29(5):621–627
19. Helman G, Bonkowsky JL, Vanderver A (2016) Neurologist comfort in the use of next-generation sequencing diagnostics: current state and future prospects. JAMA Neurol 73(6):621–622
20. Biesecker LG, Green RC (2014) Diagnostic clinical genome and exome sequencing. N Engl J Med 371(12):1170
21. Vasli N, Bohm J, Le Gras S, Muller J, Pizot C, Jost B et al (2012) Next generation sequencing for molecular diagnosis of neuromuscular diseases. Acta Neuropathol 124(2):273–283
22. Evila A, Arumilli M, Udd B, Hackman P (2016) Targeted next-generation sequencing assay for detection of mutations in primary myopathies. Neuromuscul Disord 26(1):7–15
23. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J et al (2015) Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17(5):405–424
24. Trinick J (1994) Titin and nebulin: protein rulers in muscle? Trends Biochem Sci 19(10):405–409
25. Hackman P, Vihola A, Haravuori H, Marchand S, Sarparanta J, De Seze J et al (2002) Tibial muscular dystrophy is a titinopathy caused by mutations in TTN, the gene encoding the giant skeletal-muscle protein titin. Am J Hum Genet 71(3):492–500
26. De Seze J, Udd B, Haravuori H, Sablonniere B, Maurage CA, Hurtevent JF et al (1998) The first European family with tibial muscular dystrophy outside the Finnish population. Neurology 51(6):1746–1748
27. Hackman P, Marchand S, Sarparanta J, Vihola A, Pénisson-Besnier I, Eymard B et al (2008) Truncating mutations in C-terminal titin may cause more severe tibial muscular dystrophy (TMD). Neuromuscul Disord 18(12):922–928
28. Savarese M, Maggi L, Vihola A, Jonson PH, Tasca G, Ruggiero L et al (2018) Interpreting Genetic Variants in Titin in Patients with Muscle Disorders. JAMA Neurol 75(5):557–565
29. Roberts AM, Ware JS, Herman DS, Schafer S, Baksi J, Bick AG et al (2015) Integrated allelic, transcriptional, and phenomic dissection of the cardiac effects of titin truncations in health and disease. Sci Transl Med. 7(270):270ra6
30. Golbus JR, Puckelwartz MJ, Fahrenbach JP, Dellefave-Castillo LM, Wolfgeher D, McNally EM (2012) Population-based variation in cardiomyopathy genes. Circ Cardiovasc Genet 5(4):391–399
31. Herman DS, Lam L, Taylor MR, Wang L, Teekakirikul P, Christodoulou D et al (2012) Truncations of titin causing dilated cardiomyopathy. N Engl J Med 366(7):619–628
32. Hackman P, Udd B, Bonnemann CG, Ferreiro A, Titinopathy DC (2017) 219th ENMC International Workshop Titinopathies International database of titin mutations and phenotypes, Heemskerk, The Netherlands, 29 April-1 May 2016. Neuromuscul Disord 27(4):396–407
33. Bonnemann CG (2018) Understanding titin variants in the age of next-generation sequencing-a titanic challenge. JAMA Neurol 75(5):539–540

34. Penisson-Besnier I, Hackman P, Suominen T, Sarparanta J, Huovinen S, Richard-Cremieux I et al (2010) Myopathies caused by homozygous titin mutations: limb-girdle muscular dystrophy 2J and variations of phenotype. J Neurol Neurosurg Psychiatry 81(11):1200–1202
35. Tabish AM, Azzimato V, Alexiadis A, Buyandelger B, Knoll R (2017) Genetic epidemiology of titin-truncating variants in the etiology of dilated cardiomyopathy. Biophys Rev 9(3):207–223

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