

Noteworthy Case

Centronuclear myopathy with cardiomyopathy due to recessive titinopathy

Jennifer M. Martinez-Thompson¹, MD, Thomas L. Winder, PhD², Teerin Liewluck¹, MD

¹Department of Neurology, Mayo Clinic, Rochester, MN

²Invitae Corporation, San Francisco, CA

Corresponding author:

Teerin Liewluck

200 First Street Southwest, Rochester, MN, 55905

Tel: 507-266-3544; E-mail: liewluck.teerin@mayo.edu

Running title: *TTN* centronuclear myopathy

Disclosures: None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Funding: There is no relevant funding.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/mus.26429

Abstract

N/A

Key words: cardiomyopathy, centronuclear myopathy, titin, titinopathy, *TTN*

Introduction

Titin is a protein involved in sarcomere assembly and maintenance of passive sarcomeric tension, interacting with multiple scaffolding proteins including calpain-3 and nebulin.¹ Titin gene (*TTN*) mutations, specifically truncating mutations, account for 25% of familial dilated cardiomyopathy cases.² *TTN* mutations also occur in several skeletal muscle disorders, with variable inheritance and pathologic findings, including late-onset dominant or early-onset recessive distal myopathy, limb girdle muscular dystrophy type 2J, hereditary myopathy with early respiratory failure, Emery-Dreifuss-like muscular dystrophy without cardiac involvement, early-onset myopathy with fatal cardiomyopathy, multiminicore disease with heart disease, and recessive centronuclear myopathy.^{1,3} Our case below further highlights the phenotypic variability and cardiac involvement in recessive titinopathy.

Case report

A 54-year old woman (German and Native American descent) had a 5-year history of progressive proximal weakness. Motor and cognitive development were normal. She began toe walking at age 2 and was a slow runner. Achilles tendon lengthening and scoliosis surgeries were performed in her early teens. She did well until age 35, when she was diagnosed with a dilated cardiomyopathy (ejection fraction 30%). For the past 4 years, she required nocturnal non-invasive ventilation for orthopnea. Family history was notable for two deceased siblings with similar but more severe childhood-onset symptoms (II-2 and II-3, **Figure 1**).

Neurologic examination showed a steppage gait, scoliosis, and severe symmetric limb-girdle weakness without scapular winging and sparing of the facial and ocular muscles. Creatine kinase was 40 U/L (normal < 176 U/L). Acid alpha-glucosidase was normal. Motor nerve conduction studies showed low amplitude peroneal and tibial motor responses but upper limb studies were normal. Sensory nerve conduction studies were normal. Repetitive stimulation (2-Hz) of spinal accessory and ulnar nerves showed no decrement at baseline and after 1-minute of exertion. Needle electromyography showed rapidly recruited short duration, low amplitude motor unit potentials without fibrillation potentials or myotonic discharges in proximal and axial muscles. Her vital capacity was 30% of predicted at 1.02 L (lower limit of normal 2.60 L), maximal inspiratory pressure was 49% of predicted at 37 cm H₂O (lower limit of normal 52 cm H₂O), and maximal expiratory pressure was 53% of predicted at 75 cm H₂O (lower limit of normal 101 cm H₂O). Electrocardiogram and echocardiogram while on treatment with carvedilol, digoxin, furosemide, and lisinopril for heart failure were normal. Vastus lateralis muscle biopsy performed at the time of scoliosis surgery (age 14 years) showed fiber size variation, numerous fibers with internal nuclei, and marked type 1 fiber smallness.

Next generation sequencing of 120 genes causative of myopathies and congenital myasthenic syndrome (Invitae, San Francisco, CA) showed a heterozygous splice donor variant (c.86821+2T>A) and another splice variant affecting the consensus splice donor site of exon 219 (c.40558G>C, p.Val13520Leu) in *TTN* (NM_001267550.2). Additional single variants were noted in *RAPSN* and *CLCN1* that were unlikely to account for her phenotype. No mutations were

identified in *BINI*, *CCDC78*, *DNM2*, *MTM1*, or *RYR1*. *SPEG* was not included in the sequencing panel. Her parents and remaining brother had genetic testing with results as outlined in **Figure 1**, confirming that the *TTN* variants identified in this patient were heteroallelic.

Discussion

Our patient had clinicopathologic features compatible with centronuclear myopathy.⁴ She carries compound heterozygous *TTN* mutations as mentioned above. The c.86821+2T>A mutation affects the intron 326 splice site of *TTN*, which is expected to disrupt mRNA splicing and result in an absent or truncated protein product.⁵ This mutation is known to segregate in individuals with dilated cardiomyopathy.² The c.40558G>C variant involves the terminal nucleotide of exon 219, a position in human genes regarded as part of the consensus splice donor site.⁶ The c.40558G>C variant has been reported in the heterozygous state in an individual with dilated cardiomyopathy and in compound heterozygous state in an individual with centronuclear myopathy.^{2,3}

To date, there are only 6 reported cases of histologically-confirmed centronuclear myopathy due to compound heterozygous truncating *TTN* mutations, manifesting with early childhood-onset generalized weakness, scoliosis, and respiratory insufficiency.^{2,7} The creatine kinase was normal in most cases. Cardiomyopathy was not observed in 5 recessive *TTN*-centronuclear myopathy patients first reported by Ceyhan-Birsoy et al,² thought to be due to the early age of these patients as last follow-up ranged from 5-19 years and *TTN*-associated cardiomyopathy is typically diagnosed in the fourth decade. However, Fattori et al subsequently

Accepted Article

reported a patient with centronuclear myopathy due to compound heterozygous nonsense *TTN* mutations, who was diagnosed with dilated cardiomyopathy at age 8 years.⁷ The muscle biopsy from a centronuclear myopathy patient carrying the same missense *TTN* variant as observed in our patient (c.40558G>C) and c.44816-1G>A showed loss of the C-terminal end of titin and secondary loss of calpain-3 binding on indirect immunofluorescence, implying pathogenicity of compound truncating *TTN* mutations.³

Our findings further highlight that patients with centronuclear myopathy due to recessive *TTN* mutations may develop cardiomyopathy at a varied age of onset. Close cardiac monitoring in these patients is vital, which is supported further when considering the deaths due to unknown causes of the proband's affected siblings. Intrafamilial phenotypic variability is also highlighted by earlier-onset and more severe weakness in the affected siblings.

Abbreviations: AR = autosomal recessive, CK = creatine kinase, Val = valine, Leu = leucine, *TTN* = titin, *RAPSN* = rapsyn

References

1. Savarese M, Sarparanta J, Vihola A, Udd B, Hackman B. Increasing role of titin mutations in neuromuscular disorders. *J Neuromuscul Dis* 2016; 3(3):293-308.
2. Herman DS, Lam L, Taylor MRG, Wang L, Teekakirikul P, Christodoulou D et al. Truncations of titin causing dilated cardiomyopathy. *NEJM* 2012; 366(7): 619-628.
3. Ceyhan-Birsoy O, Agrawal PB, Hidalgo C, Schmitz-Abe K, DeChene ET, Swanson LC et al. Recessive truncating titin gene, TTN, mutations presenting as centronuclear myopathy. *Neurology* 2013; 81:1205-1214.
4. Jungbluth H, Gautel M. Pathogenic mechanisms in centronuclear myopathies. *Front Aging Neurosci* 2014; 6: 1-11.
5. Roberts AM, Ware JS, Herman DS, Schafer S, Baksi J, Bick AG et al. Integrated allelic, transcriptional, and phenomic dissection of the cardiac effects of titin truncations in health and disease. *Sci Transl Med* 2015; 7(270): 1-14.
6. Buratti E, Chivers M, Kralovicova J, Romano M, Baralle M, Krainer AR et al. Aberrant 5' splice sites in human disease genes: mutation pattern, nucleotide structure and comparison of computational tools that predict their utilization. *Nucleic Acids Research* 2007; 35 (13): 4250-4263.
7. Fattori F, Maggi L, Bruno C, Cassandrini D, Codemo V, Catteruccia M et al. Centronuclear myopathies: genotype-phenotype correlation and frequency of defined genetic forms in an Italian cohort. *J Neurol* 2015; 262(7):1728-1740.

Figure Legend

Figure 1. Family pedigree and proband. (A) The arrow indicates the proband (II-1). A brother (II-2) had childhood cognitive delay, severe weakness with retained ambulatory capability, an unspecified heart murmur, and scoliosis requiring surgery. He died at age 20 of unknown causes. A sister (II-3) had severe early childhood-onset weakness impairing ambulation, scoliosis, a heart murmur, and Achilles tendon contractures requiring lengthening surgery. She died at age 17 of unknown causes. Both affected brother (II-2) and sister (II-3) were diagnosed with muscular dystrophy, but never underwent genetic testing or muscle biopsy. The proband's parents, a brother (II-4), and a son (III-1) are healthy. Asterisks denote individuals who underwent genetic testing with the corresponding variants in the titin-encoding gene (*TTN*) listed.

