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A Titin Truncating Variant Causing a Dominant Myopathy With Cardiac Involvement in a Large Family

The Exception That Proves the Rule

Kristl G. Claeys, MD, PhD, Marco Savarese, PhD, Per Harald Jonson, PhD, Veerle Goosens, MD, Ana Topf, PhD, Anna Vihola, PhD, Volker Straub, MD, PhD, and Bjarne Udd, MD, PhD

Correspondence
Prof. Dr. Claeys
kristl.claeys@uzleuven.be

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Abstract

Background

Titin truncating variants (TTNtvs) have been repeatedly reported as causative of recessive but not dominant skeletal muscle disorders.

Objective

To determine whether a single heterozygous nonsense variant in *TTN* can be responsible for the observed dominant myopathy in a large family.

Methods

In this case series, all available family members (8 affected and 6 healthy) belonging to a single family showing autosomal dominant inheritance were thoroughly examined clinically and genetically.

Results

All affected family members showed a similar clinical phenotype with a combination of cardiac and skeletal muscle involvement. Muscle imaging data revealed titin-compatible hallmarks. Genetic analysis revealed in all affected patients a nonsense *TTN* variant c.70051C>T p.(Arg23351*), in exon 327. RNA sequencing confirmed the lack of complete nonsense-mediated decay, and protein studies convincingly revealed expression of a shortened titin fragment of the expected size.

Discussion

We conclude that a single heterozygous nonsense variant in titin occasionally can cause a dominant myopathy as shown in this large family. Therefore, monoallelic titin truncating variants should be considered as possible disease-causing variants in unsolved patients with a dominant myopathy. However, large segregation studies, muscle imaging, and RNA and protein assays are needed to support the clinical and genetic interpretation.

Introduction

Titin truncating variants (TTNtvs) are identified in around 0.5%–1% of the general population.[1] Heterozygous TTNtvs have been identified in patients with dilated cardiomyopathy (DCM), suggesting their role in increasing the DCM disease risk.[2]

From the Department of Neurology (K.G.C.), University Hospitals Leuven; Department of Neurosciences (K.G.C.), Laboratory for Muscle Diseases and Neuropathies, KU Leuven, and Leuven Brain Institute (LBI), Belgium; Folkhälsan Research Center and Medicum (M.S., P.H.J., A.V., B.U.), University of Helsinki, Finland; Department of Radiology (V.G.), University Hospitals Leuven, Belgium; John Walton Muscular Dystrophy Research Centre (A.T., V.S.), Translational and Clinical Research Institute, Newcastle University and Newcastle Hospitals NHS Foundation Trust, Newcastle upon Tyne, United Kingdom; Neuromuscular Research Center (B.U.), Department of Neurology, Tampere University and University Hospital; and Department of Neurology (B.U.), Vaasa Central Hospital, Finland.

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In recent years, an increasing number of patients with biallelic TTNtv resulting in recessive myopathies with antenatal, congenital, infantile, or later onset have been reported.[3-6] So far, heterozygous carrier parents and relatives of the identified patients with biallelic TTNtv have always been unaffected.[6] At the same time, despite multiple attempts, the expression of truncated titin proteins in skeletal muscles has usually not been observed, corroborating the hypothesis that alleles with TTNtv are mostly null and not disease causing in a heterozygous state. On the contrary, truncated proteins seem to be expressed in cardiac cells in some cases, and this may partly explain the increased prevalence of TTNtv in patients with DCM.[7,8]

In this article, we report a large family with a dominantly inherited myopathy. Extensive clinical, genetic, and molecular characterization showed that the identified heterozygous nonsense variant in *TTN* plays a causative role in the disease etiology.

Methods

A total of 8 affected and 6 healthy family members (Figure 1A) were recruited into the study. Clinical data were collected at the Department of Neurology, University Hospitals Leuven. Whole-body muscle MRI (MRI; 1.5T) and muscle biopsies followed standard procedures.

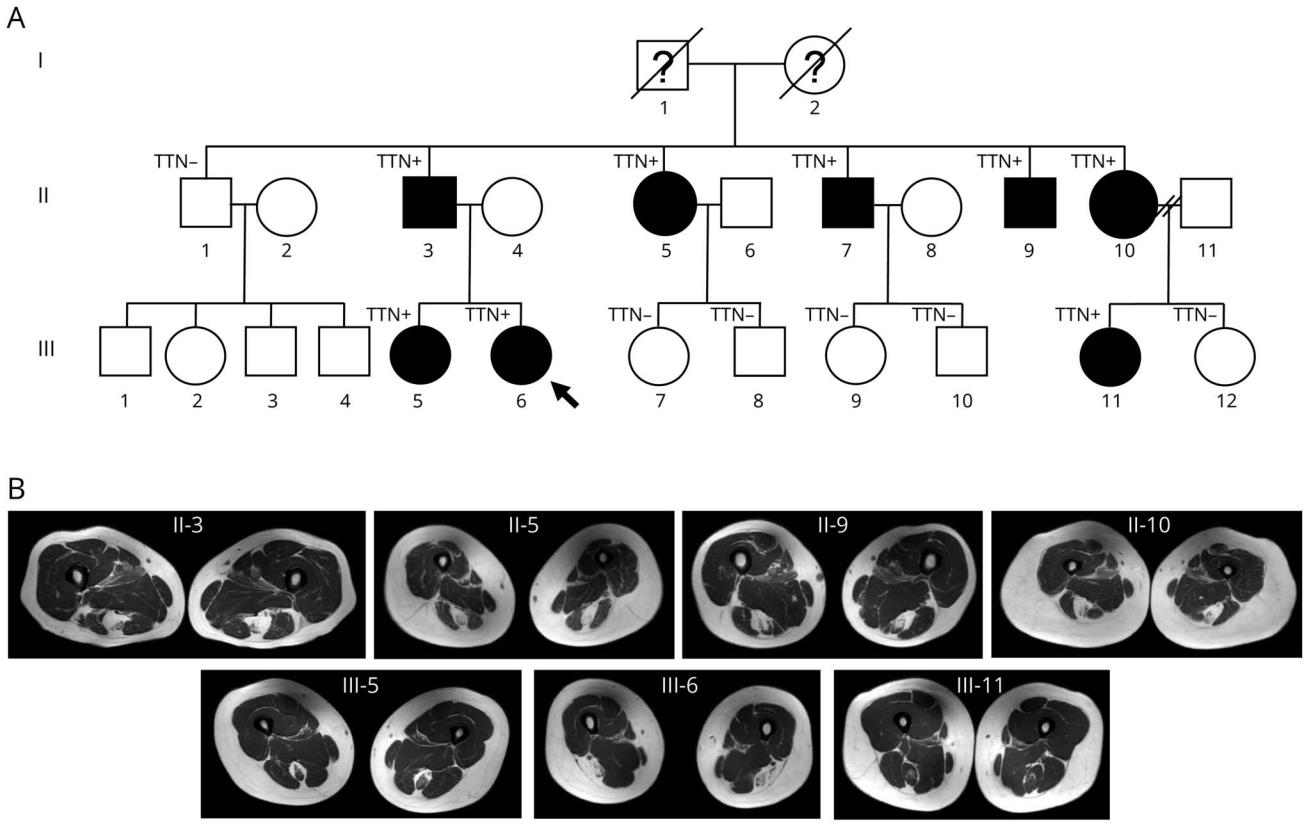
Genomic DNA was extracted from blood by phenol/chloroform purification. DNA of the proband (III.6) and affected family member II.5 were analyzed as described previously.[9] DNA of affected family members II.3, II.10, and III.5 were analyzed by exome sequencing at BGI Genomics (Denmark). In addition, long-read genome sequencing was performed within the Solve-RD project in the proband III.6, her affected father II.3, her unaffected mother II.4, and her affected sister III.5. Finally, whole-genome sequencing was performed in patients III.5 and III.11 at CeGaT (Germany).

Segregation analysis of the *TTN* variant was performed by PCR and Sanger sequencing. RNA extracted from the proband's skeletal muscle biopsy was analyzed by RNA sequencing after ribodepletion, and titin protein expression was examined using 1% agarose gels.[10]

Standard Protocol Approvals, Registrations, and Patient Consents

The study was approved by the ethics committee of the Universities of Helsinki and Leuven and performed in accordance

Figure 1 Pedigree of the Family and Muscle MRI Findings



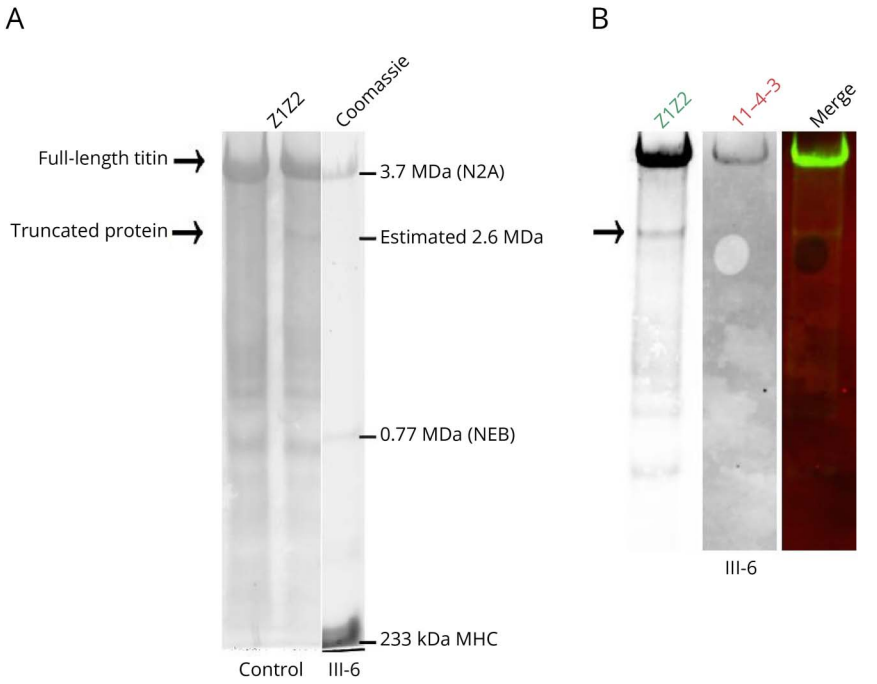
(A) Black symbols indicate affected patients (circles: women, squares: men). The arrow points to the proband of the family. "TTN+" refers to the patients carrying the *TTN* truncating variant, whereas "TTN-" indicates the absence of the variant. '?' indicates that the clinical phenotype is unknown. (B) An image of muscle MRI at the level of the thighs is shown for the affected patients. The number corresponds to the number in the pedigree.

Table Clinical Features of Patients Indicated in the Pedigree

Patient	Symptom onset	First symptoms	Current muscle weakness (all adults)/muscle MRI findings	CK	EMG	Current cardiac function	Current respiratory function
II.3	Childhood	Scoliosis	Proximal weakness; arm abductors; neck flexor weakness and atrophy; abdominal weakness; lumbar hyperlordosis; muscle MRI shows fatty replacement in ST (4), BFS (3), AL (2), GM (2), ES (3), ABD (3), SC (3), and BB (2)	100	—	LVEF 50%; ECG and 24h-Holter-ECG normal; cyclo-ergometry normal; cardiac MRI no fibrosis	FVC 57% sitting
II.5	Childhood (skeletal)/40s (cardiac)	Difficulties in gymnastics at school: handstands, rope climbing, pumping, running, and jumping; at adult age: dilated cardiomyopathy (LVEF 21%) with dyspnea, orthopnea, and palpitations	Proximal weakness; hip flexors, knee flexors, arm abductors, and elbow flexors; neck flexor weakness; abdominal weakness; lumbar hyperlordosis; Gowers sign; muscle MRI shows fatty replacement in ST (4), GM (3), SO (3), ES (3), ABD (2), SC (3), and BB (2)	127	Myogenic pattern (iliopsoas) without spontaneous activity	LVEF 45%; ECG and 24h-Holter-ECG normal; cardiac MRI no fibrosis	FVC 92% sitting
II.7	40s	Dilated cardiomyopathy (LVEF 24%) with dyspnea, orthopnea, and malleolar edema	No muscle weakness; muscle imaging not performed	54	Myogenic pattern proximal LL without spontaneous activity	LVEF 35% (dilated)	FVC 40% sitting
II.9	Childhood (skeletal)/50s (cardiac)	Scoliosis; at adult age: dilated congestive cardiomyopathy (LVEF 25%) with dyspnea, orthopnea, palpitations, and tachycardia	No muscle weakness; muscle MRI shows fatty replacement in ST (4), AL (2), ES (3), SC (3), and BB (3)	89	Normal	LVEF 40–45%; ECG and 24h-Holter-ECG normal; cyclo-ergometry normal; cardiac MRI no fibrosis	—
II.10	50s	Dilated cardiomyopathy (LVEF 41%) with palpitations and tachycardia	Proximal weakness hip flexors; neck flexor weakness; abdominal weakness; lumbar hyperlordosis; muscle MRI shows fatty replacement in ST (4), AL (3), SM (2), GM (2), ES (2), ABD (2), SC (3), and BB (2)	73	—	LVEF 55%; ECG and 24h-Holter-ECG normal; cardiac MRI no fibrosis	FVC 72% sitting, 66% supine
III.5	Childhood	Difficulties in gymnastics at school: could not lift head from the floor in supine position with impossibility to perform sit-ups and difficulties to lift head during swimming, handstands, rope climbing, pumping, running, and jumping	Proximal weakness; hip flexors, arm abductors, and elbow flexors; neck flexor weakness; lumbar hyperlordosis; muscle MRI shows fatty replacement in ST (3) and ES (3)	54	—	LVEF 45%; ECG normal; cyclo-ergometry normal; cardiac MRI no fibrosis	—
III.6 (proband)	20s	Postpartum dilated biventricular cardiomyopathy without myocarditis (LVEF 10%) (first child) with dyspnea and malleolar edema	Proximal weakness; hip flexors, knee extensors, and arm abductors; neck flexor weakness and atrophy; Gowers sign; lumbar hyperlordosis; nasal speech; clinodactyly of fingers V; muscle MRI shows fatty replacement in ST (4), BFL (4), AL (2), ES (3), and SC (2)	38	Myogenic pattern (iliopsoas, deltoid) without spontaneous activity	LVEF 45%; ECG and 24h-Holter-ECG normal; cyclo-ergometry normal; cardiac MRI no fibrosis	FVC 61% sitting, 56% supine
III.11	asymptomatic	asymptomatic	Asymptomatic; muscle MRI shows fatty replacement in ST (2)	92	—	LVEF 60%; ECG normal; cardiac MRI normal without fibrosis	—

Abbreviations: — = not performed; 24h-Holter-ECG = 24 h ECG measurement; ABD = abdominal muscles; AL = adductor longus; BB = biceps brachii; BFL = biceps femoris long head; BFS = biceps femoris short head; CK = creatine kinase (upper normal value for men 190 U/l and for women 170 U/l); ECG = electrocardiography; ES = erector spinae; FVC = forced vital capacity; GM = medial gastrocnemius; LL = lower limbs; LVEF = left ventricular ejection fraction; SC = subscapular; SM = semimembranosus; SO = soleus; ST = semitendinosus.
The numbers between brackets refer to the Mercuri semiquantitative score for muscle involvement on MRI (score 4 indicates most severely affected). The patient numbers are presented in the pedigree in Figure 1.

Figure 2 Western Blot From VAGE Showing Full-Length Titin and the Truncated Protein From the Mutant Allele in the Proband



(A) The absence of the truncated band in a healthy human control (lane 1), whereas it is visible in III.6 (lane 2) using the N-terminal antibody Z1Z2. Lane 2 was cut to allow Coomassie staining for the size marker using the molecular weight of titin N2A isoform (N2A), nebulin (NEB) and myosin heavy chain (MHC), and the estimated weight of the truncated titin band. (B) The truncated protein is not stained using the C-terminal antibody 11-4-3, but again with Z1Z2. The theoretical molecular weight for the NM_001267550.1:c.70051C>T p.(Arg23351*) isoform is exactly 2.6 MDa as predicted from the size markers.

with the Declaration of Helsinki. Written informed consent was obtained from all patients.

Data Availability

The data that support the findings of this study are available from the corresponding author on reasonable request.

Results

The proband (Figure 1A; III.6) manifested dyspnea and edema because of postpartum cardiomyopathy, 8 weeks after birth of her first child, with rapid recuperation of left ventricular ejection fraction (LVEF) to 50% (normal ≥60%) with medication. She consulted 2 years later because of progressive proximal muscle weakness, resulting in Trendelenburg gait and a Gowers sign (Table).

EMG in iliopsoas and deltoid muscles revealed a myogenic pattern without spontaneous activity. A vastus lateralis muscle biopsy showed mildly increased variability in muscle fiber diameter, increased number of internalized nuclei, slightly moth-eaten pattern, and an increased type 1 fiber proportion, compatible with titinopathy. A recent cardiac assessment showed a mildly reduced LVEF of 45%. Cardiac MRI excluded endocardial fibrosis. Serum creatine kinase (CK) was normal. T1-weighted muscle MRI showed fatty replacement of semitendinosus and biceps femoris muscles (Figure 1B, Table).

A similar clinical phenotype with combined cardiac and skeletal muscle involvement was present in the proband's parent (II.3) and 4 siblings of the parent (II.5, II.7, II.9, II.10) and the proband's sibling (III.5). 1 adult cousin (III.11) was subjectively asymptomatic but showed fatty transformation in semitendinosus muscles bilaterally on MRI similar to the affected relatives (Figure 1B, Table) and had normal cardiac examinations (Table). She is the youngest affected patient, which might explain the current absence of subjective symptoms. Findings on MRI revealed a pattern of muscle involvement compatible with titinopathy, with semitendinosus muscle most severely affected in all patients and the only affected muscle in the asymptomatic patient (Figure 2B, Table).

Patients II.1, III.7, III.8, III.9, III.10, and III.12 were asymptomatic and had normal clinical examination and CK levels. Whole-body muscle MRI performed in 2 of them (III.7, III.12) and echocardiography in 5 (II.1, III.7, III.8, III.9, III.10) did not show abnormalities.

Exome sequencing of the proband's DNA revealed a nonsense *TTN* variant, absent in the control population (gnomAD v.4.0):NM_001267550.1:c.70051C>T p.(Arg23351*), in exon 327, corresponding to titin A-band.

Segregation analysis in 14 family members proved that the *TTN* nonsense variant co-segregated with the disease (Figure 1A). Long-read genome sequencing in proband III.6, her affected father II.3, her unaffected mother II.4, and her affected sister III.5 and whole-genome sequencing in patients III.5 and III.11 did not reveal an additional genetic cause.

RNA sequencing confirmed that the *TTN* variant did not affect splicing and ruled out complete nonsense-mediated decay (NMD), as shown by a slightly reduced expression of the mutated allele (35%). A full-length titin Western blot detected a band of reduced size, compatible with the expected size of the truncated protein (Figure 2).

Discussion

TTNtvs refer to different classes of variants that may elicit nonsense-mediated RNA decay or heterogeneous downstream effects.[5] Although TTNtvs have a cumulative frequency up to 1% in the general population,[1] their exact location seems to play a crucial role in determining a possible effect on the corresponding titin product, as demonstrated in a recent cardiomyopathy study.[7] Thereby, the identification of a single heterozygous truncating variant co-segregating with a dominant cardiomyopathy in this family was not surprising, but the concomitant skeletal myopathy as shown by the extensive muscle MRI changes was unforeseen.

The clinical history of the affected family members and, above all, the muscle imaging data showing *TTN*-compatible hallmarks confirmed the diagnosis of a dominant titinopathy.

In total, 4 previous reports have described the same c.70051C>T variant in *TTN* as a cause of DCM, but none have reported skeletal muscle weakness. This might be explained by the skeletal muscle weakness occurring later than DCM or being relatively mild and, therefore, not being recognized, especially if the patient consults a cardiologist and no clinical neurologic examination is performed.[11-14]

Although we have previously reported cases of pseudodominance and of digenic mechanisms involving titin,[15] we cannot completely exclude an additional causative variant or a digenism in this family. However, the large family with complete co-segregation data, the patients' clinical picture and muscle MRI findings, and the unexpected presence of a truncated protein (never seen so far in other patients with myopathy with TTNtv, irrespective of their location) point toward a primary causative role of the identified TTNtv.

NMD efficiency is strongly reduced in exceptionally long exons. The reported variant is located in the 5′ region of the largest *TTN* exon (exon 327 with 17,106 nucleotides). Heterozygous variants in the same region of the exon have been identified in patients with DCM [16] without data on skeletal muscle involvement. Further studies are needed to understand how NMD is regulated in such a complex and long transcript and how the location of premature stop codons along the long transcript affects it. However, although we were able to analyze only a single muscle biopsy, the presence of a shortened titin fragment of the expected size suggests a gain-of-function mechanism that explains the dominant effect of the variant.

Our study suggests that heterozygous *TTN*tv may occasionally cause autosomal dominant forms of titinopathy presenting with cardiac and skeletal muscle symptoms. *TTN* is, therefore, a candidate gene for unsolved patients with a dominant myopathy beyond the previously described dominant titinopathies: hereditary myopathy with early respiratory failure and tibial muscular dystrophy.

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Disclosure

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Appendix Authors

Name	Location	Contribution
Kristl G. Claeys, MD, PhD	Department of Neurology, University Hospitals Leuven; Department of Neurosciences, Laboratory for Muscle Diseases and Neuropathies, KU Leuven, and Leuven Brain Institute (LBI), Belgium	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data
Marco Savarese, PhD	Folkhälsan Research Center and Medicum, University of Helsinki, Finland	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data
Per Harald Jonson, PhD	Folkhälsan Research Center and Medicum, University of Helsinki, Finland	Drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data
Veerle Goosens, MD	Department of Radiology, University Hospitals Leuven, Belgium	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data

Continued

Appendix *(continued)*

Name	Location	Contribution
Ana Topf, PhD	John Walton Muscular Dystrophy Research Centre, Translational and Clinical Research Institute, Newcastle University and Newcastle Hospitals NHS Foundation Trust, Newcastle upon Tyne, United Kingdom	Drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data
Anna Vihola, PhD	Folkhälsan Research Center and Medicum, University of Helsinki, Finland	Drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data
Volker Straub, MD, PhD	John Walton Muscular Dystrophy Research Centre, Translational and Clinical Research Institute, Newcastle University and Newcastle Hospitals NHS Foundation Trust, Newcastle upon Tyne, United Kingdom	Drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data
Bjarne Udd, MD, PhD	Folkhälsan Research Center and Medicum, University of Helsinki; Neuromuscular Research Center, Department of Neurology, Tampere University and University Hospital; Department of Neurology, Vaasa Central Hospital, Finland	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data

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