Congenital myopathy associated with the triadin knockout syndrome

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ABSTRACT

Objective: Triadin is a component of the calcium release complex of cardiac and skeletal muscle. Our objective was to analyze the skeletal muscle phenotype of the triadin knockout syndrome.

Methods: We performed clinical evaluation, analyzed morphologic features by light and electron microscopy, and immunolocalized triadin in skeletal muscle.

Results: A 6-year-old boy with lifelong muscle weakness had a triadin knockout syndrome caused by compound heterozygous null mutations in triadin. Light microscopy of a deltoid muscle specimen shows multiple small abnormal spaces in all muscle fibers. Triadin immunoreactivity is absent from type 1 fibers and barely detectable in type 2 fibers. Electron microscopy reveals focally distributed dilation and degeneration of the lateral cisterns of the sarcoplasmic reticulum and loss of the triadin anchors from the preserved lateral cisterns.

Conclusions: Absence of triadin in humans can result in a congenital myopathy associated with profound pathologic alterations in components of the sarcoplasmic reticulum. Why only some triadin-deficient patients develop a skeletal muscle phenotype remains an unsolved question.

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GLOSSARY

CASQ = calsequestrin; **DHPR** = dihydropyridine receptor; **RyR** = ryanodine receptor; **SR** = sarcoplasmic reticulum.

Triadin knockout syndrome is a recessively inherited cardiac arrhythmia syndrome manifesting with extensive T-wave inversion in precordial leads V₁–V₄ with consistent or transient QT prolongation, exercise-induced cardiac arrest in early childhood, and possible muscle weakness.¹

Triadin is a critical component of the Ca²⁺ release complex responsible for excitation-contraction coupling. Alternative splicing *TRDN* results in 3 differentially expressed isoforms in cardiac and skeletal muscle. A 32 kDa isoform is expressed in heart and the 95 and 51 kDa isoforms express predominantly in skeletal muscle.^{2,3}

The Ca²⁺ release complex is positioned where the lateral cisterns of the sarcoplasmic reticulum (SR) abut on the transverse tubules (T-tubules). It includes the voltage-gated Ca²⁺ channels in the T-tubule, known as dihydropyridine receptors (DHPRs), and Ca²⁺ release channels in the lateral SR cisterns, known as the ryanodine receptors (RyRs), in skeletal muscle (figure 1A). The RyRs are arrayed on the surface of lateral cisterns facing the T-tubule and align with the DHPRs. The lateral cisterns contain calsequestrin (CASQ), a high-capacity calcium-binding protein. Propagation of the muscle fiber action potential into the T-tubules changes conformation of the DHPRs; this is transmitted to the RyRs, effecting opening of the Ca²⁺ release channels, allowing Ca²⁺ stored by CASQ to diffuse into the myoplasm and activate the contractile machinery. Subsequently, a Ca²⁺-activated ATPase of the SR pumps Ca²⁺ back into the SR, where it is restored by CASQ.⁴

Triadin molecules self-assemble from periodic anchors between RyRs and CASQ in the lateral cisterns (figure 1A)³ and interact with nearby microtubules.²

Supplemental data
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Figure 1 Schematic diagram of T-tubule associated with sarcoplasmic reticulum (SR) components and light microscopy findings in triadin myopathy

(A) Schematic diagram. Periodically positioned ryanodine receptors (RyRs) appear on the surface of the lateral SR cistern facing the T-tubule. The triadin anchors associated with the RyRs extend into the lateral cistern containing calsequestrin (CASQ), where they are interconnected by calsequestrin condensates. (B) Phase optic image of unstained frozen section of patient muscle reveals multiple small abnormal spaces within the fibers. (C) Trichrome-stained frozen section displays similar small spaces as well as small canaliculi in the fibers. Patient (D) and control muscle (E) specimens immunoreacted for triadin. Note markedly attenuated triadin expression in patient muscle. Schematic diagram in panel A is based in part on electron microscopic image of a triad in figure 2C in reference 4. Bars: B, 50 μ m; C, 25 μ m; D, 20 μ m; E, 50 μ m.

Clinical data. We describe the clinical findings and document for the first time the skeletal muscle pathology in a patient with triadin knockout syndrome caused by a maternally derived frameshift mutation, p.K147fs*0, and a paternally inherited frameshift mutation, p.N9fs*5 in *TRDN*.¹ The patient, now 6 years of age, had bradycardia in utero with ECGs documenting borderline prolonged QT intervals and deeply inverted precordial T waves in V₁–V₄. At age 20 months, he had cardiac arrest associated with ventricular fibrillation. He was diagnosed with a possible long QT syndrome and treated with a β -blocker and an implanted cardioverter defibrillator. A year later, this prevented an episode of exertion-induced ventricular fibrillation. Skeletal muscle involvement was first suspected because he was hypomotile in utero and hypoactive after birth. He

sat at age 6 months and walked at age 14 months but fatigued easily, had difficulty getting dressed, could walk only short distances, and was unable to participate in strenuous activities. Manual muscle testing at age 5 years showed mild to moderate weakness of the suprascapular, pectoralis major, deltoid, finger extensor, and interossei muscles, moderate weakness of the iliopsoas, anterior tibial, and toe extensor muscles. The facial, extraocular, and eyelid elevator muscles were spared. The tendon reflexes were absent at rest and barely elicited with reinforcement. Clinical electrophysiology studies revealed normal nerve conduction velocities. A neuromuscular transmission defect was excluded by no decrement of the evoked muscle potential on repetitive nerve stimulation and normal single-fiber EMG. Needle EMG examination of upper and lower limb muscles revealed no abnormality. The serum creatine kinase level fell in the normal range.

METHODS Standard protocol approval, registrations, and patient consents. All human studies were approved by the institutional review board of the Mayo Clinic. The muscle biopsy studies were obtained for diagnosis with parental consent.

Laboratory studies. Fresh frozen sections of the muscle specimen were processed for routine histochemical studies and a separate glutaraldehyde-fixed specimen was prepared for electron microscopy. Triadin was immunolocalized with a polyclonal rabbit antibody (NBP1-87417, Novus Biologicals, Littleton, CO) and visualized with a biotinylated donkey anti-rabbit antibody (Jackson ImmunoResearch, West Grove, PA) and a Vector ABC kit (Vector Laboratories, Burlingame, CA).

The compound heterozygous frameshifting p.N9fs and p.K147fs*0 mutations were identified previously in our patient following a mutational analysis of *TRDN* using DNA Sanger sequencing.¹

RESULTS Muscle biopsy studies. Routine histochemical studies of frozen sections of the left deltoid muscle showed 3 regenerating fibers among several thousand. An unstained frozen section viewed under phase optics revealed myriad small circular abnormal spaces in most muscle fibers (figure 1B). In a slightly obliquely cut trichrome-stained section, some abnormal spaces appeared as canaliculi associated with dot-like purple membranous material (figure 1C). Immunostains for triadin of a normal control muscle showed that some fibers reacted more strongly than others (figure 1E). Triadin expression was markedly attenuated in the patient's muscle compared to a normal control muscle with only slight immunoreactivity persisting in one set of muscle fibers (figure 1D). The more reactive patient and control fibers were deemed to be histochemically type 2 because comparison of adjacent triadin and ATPase reacted sections of a normal control muscle showed that triadin is more

Figure 2 Ultrastructural findings in skeletal muscle

(A–C) Dilated and degenerate components of the lateral cisterns, some of which abut on T-tubules (T) in the plane of sectioning. In A and C, the abnormal spaces also contain ~60-nm-wide bands studded with electron-dense particles that might represent condensed remnants of calsequestrin (asterisks). Bars: A, 0.8 μm ; B and C, 0.6 μm .

highly expressed in type 2 than type 1 fibers (data not shown).

Electron microscopy studies revealed focally distributed degenerative changes involving the SR. In some regions, the T-tubules were flanked by markedly enlarged abnormal spaces that were empty, or contained myeloid structures (figure 2, A and B), or displayed irregularly circular ~60-nm-wide bands studded with minute electron-dense particles (asterisks in figure 2, A and C). Some muscle fibers showed subsarcolemmal aggregates of degenerate vesicular profiles intermingled with glycogen particles and mitochondria (figure e-1 at Neurology.org). Some fiber regions also harbored preserved lateral SR cisterns flanking T-tubules in triads (figure 3, B and C). We compared these triads with those found in normal muscle (figure 3A) and searched for the triadin anchors projecting into the lateral cisterns in both. In favorable sections of normal muscle, we detected triadin anchors projecting into the lumen of the lateral cisterns (arrow in figure 3A) but could not resolve the RyRs in the gap between the T-tubules and the lateral cisterns. In the patient's muscle, the RyR1 profiles were resolved but no triadin-like formations were detected in the lateral cisterns. The lateral cisterns were dilated and contained finely granular material (figure 3, B and C).

DISCUSSION Previous studies of triadin knockout mice revealed several functional and structural abnormalities. The size of the lateral cisterns^{5,6} as well as their CASQ^{6,7} and Ca^{2+} content were reduced,⁶ and CASQ appeared outside the SR. The contacts between the junctional SR and the T-tubules were decreased, colocalization of the DHPR and the RyR was attenuated, and the RyR and junctin contents of the junctional SR were reduced.⁷ In our patient, there was marked focally distributed destruction of the lateral cisterns associated with disrupted contacts with the T-tubules. These findings predict reduced buffering of Ca^{2+} entering the muscle fibers, increased

Figure 3 Triads in normal and triadin-deficient muscle

(A) In normal muscle, triadin anchors appear on inner surface of lateral cisterns closest to the T-tubule-lateral cistern interspace. The ryanodine receptors (RyRs) are not well-resolved. (B, C) In triadin-deficient triads, the RyRs are resolved but no triadin anchors appear on inner surface of lateral cisterns. Bar = 0.1 μm for each panel.

myoplasmic Ca^{2+} levels, and impaired contraction of myofibrils due to focal degeneration of the SR.

Considering that mutations causing the triadin knockout syndrome cause loss of both cardiac and skeletal muscle triadin isoforms, a skeletal muscle as well as a cardiac phenotype is anticipated. In fact, decreased muscle strength was observed in triadin knockout mice.⁶ However, while nearly all patients with triadin knockout syndrome have a severe cardiac phenotype, muscle weakness is variable. Among our 5 unrelated patients described previously¹ and a total of 8 additional triadin-null patients from 4 unrelated families described by others,^{8–10} only 4 patients had muscle weakness, including the patient herein reported. Why only some triadin-deficient patients show a skeletal muscle phenotype even when their sibling harboring identical *TRDN* mutations shows no signs of muscle weakness remains an unanswered question.

AUTHOR CONTRIBUTIONS

Andrew G. Engel: study concept and design, data acquisition, analysis and interpretation, and manuscript preparation. Keely Redhage: data acquisition, critical revision of the manuscript for important intellectual content. David J. Tester: data acquisition, critical revision of the manuscript for important intellectual content. Michael J. Ackerman: study concept and design, data acquisition, critical revision of the manuscript for important intellectual content. Duygu Selcen: study concept and design, data acquisition, analysis and interpretation, and manuscript preparation.

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A. Engel has received an NIH grant (NS6277). K. Redhage reports no disclosures relevant to the manuscript. D. Tester is receiving royalties from Transgenomic. M. Ackerman is receiving consultant fees from Boston Scientific, Gilead Sciences, Invitae, Medtronic, MyoKardia, and St. Jude Medical, and royalties from Transgenomic, and is supported by Federal awards (HD042569, HL124319, and HL125918). D. Selcen reports no disclosures relevant to the manuscript. Go to Neurology.org for full disclosures.

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