Homozygous/Compound Heterozygous Triadin Mutations Associated With Autosomal-Recessive Long-QT Syndrome and Pediatric Sudden Cardiac Arrest

Elucidation of the Triadin Knockout Syndrome

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**Background**—Long-QT syndrome (LQTS) may result in syncope, seizures, or sudden cardiac arrest. Although 16 LQTS-susceptibility genes have been discovered, 20% to 25% of LQTS remains genetically elusive.

**Methods and Results**—We performed whole-exome sequencing child–parent trio analysis followed by recessive and sporadic inheritance modeling and disease-network candidate analysis gene ranking to identify a novel underlying genetic mechanism for LQTS. Subsequent mutational analysis of the candidate gene was performed with polymerase chain reaction, denaturing high-performance liquid chromatography, and DNA sequencing on a cohort of 33 additional unrelated patients with genetically elusive LQTS. After whole-exome sequencing and variant filtration, a homozygous p.D18fs*13 TRDN-encoded triadin frameshift mutation was discovered in a 10-year-old female patient with LQTS with a QTc of 500 milliseconds who experienced recurrent exertion-induced syncope/cardiac arrest beginning at 1 year of age. Subsequent mutational analysis of TRDN revealed either homozygous or compound heterozygous frameshift mutations in 4 of 33 unrelated cases of LQTS (12%). All 5 TRDN-null patients displayed extensive T-wave inversions in precordial leads V1 through V4, with either persistent or transient QT prolongation and severe disease expression of exercise-induced cardiac arrest in early childhood (≤3 years of age) and required aggressive therapy. The overall yield of TRDN mutations was significantly greater in patients ≤10 years of age (5 of 10, 50% vs 0 of 24, 0%; P=0.0009).

**Conclusions**—We identified TRDN as a novel underlying genetic basis for recessively inherited LQTS. All TRDN-null patients had strikingly similar phenotypes. Given the recurrent nature of potential lethal arrhythmias, patients fitting this phenotypic profile should undergo cardiac TRDN genetic testing.

**Key Words:** arrhythmias, cardiac genetics, heart arrest, humans, long QT syndrome, pediatrics

Sudden cardiac death is a major worldwide public health burden, with an estimated annual incidence ranging from 180,000 to 450,000 in the United States and as much as 3.7 million deaths worldwide. Pediatric sudden cardiac arrest (SCA) has a devastating and profound societal impact. Approximately 3000 infants die suddenly and unexpectedly each year before reaching 1 year of age, and another 2000 to 5000 young people between 1 and 35 years die after an SCA yearly. Those who survive an SCA are often left with neurological sequelae.

**Clinical Perspective on p 2060**

Long-QT syndrome (LQTS), with an estimated prevalence of 1 in 2500, is a major and often preventable cause of SCA in the young. LQTS, characterized by delayed ventricular cardiomyocyte repolarization and cardiac action potential prolongation that may present as a prolonged QT interval on a 12-lead surface ECG, may manifest as syncope, seizures, or SCA typically after a precipitating event such as exertion, extreme emotion, or auditory stimuli or even while at rest. Identifying and understanding the genetic origin can have a profound lifesaving impact on the overall clinical management and prophylactic treatment of a patient with LQTS.

LQTS is most often inherited as an autosomal-dominant trait; however, sporadic and autosomal-recessive inheritance patterns have been reported. LQTS is characterized as a cardiac channelopathy with the majority of cases caused by mutations within 3 genes (KCNQ1, KCNH2, and SCN5A) that
encode for critical ion channel α-subunits responsible for the cardiac action potential. However, ≈20% of patients with clinically definite LQTS remain genetically elusive.7

In this study, we performed whole-exome sequencing (WES) to identify a novel genetic explanation for an LQTS pedigree with a presumed sporadic/autosomal-recessive inheritance pattern and a subsequent mutational analysis of the novel candidate gene on an additional cohort of unrelated, genetically elusive patients with LQTS. Collectively, the phenotype for this novel, potentially lethal syndrome, called triadin knockout syndrome, is detailed.

Methods

Sporadic/Autosomal-Recessive LQTS Pedigree

A black family with presumed sporadic or autosomal-recessive LQTS was referred to the Mayo Clinic Windland Smith Rice Sudden Death Genomics Laboratory for further research-based genetic testing after negative commercially available genetic testing for LQTS. The index case was a 10-year-old girl who first experienced syncope at 1 year of age while riding her tricycle and then again at 2 years of age while dancing. Both parents were considered unaffected with normal cECGs and negative personal and family histories of cardiac-related events. After they provided written informed consent for this Mayo Clinic Institutional Review Board–approved study, blood was collected for all 3 family members, and genomic DNA was isolated.

Whole-Exome Sequencing

WES and subsequent variant annotation were performed on genomic DNA derived from the symptomat index case, unaffected father, and unaffected mother by the Mayo Clinic Advanced Genomics Technology Center and Bioinformatics Core facilities. Paired-end libraries were prepared following the manufacturer’s protocol (Agilent) with the Brady liquid handler from Agilent. Briefly, 1 to 3 μg genomic DNA was fragmented to 150 to 200 bp with the Covaris E210 sonicator. The ends were repaired, and an A base was added to the 3′ ends. Paired-end Index DNA adaptors (Agilent) with a single T-base overhang at the 3′ ends were ligated, and the resulting constructs were purified by use of AMPure SPRI beads (Agencourt). The adapter-modified DNA fragments were enriched by 4 cycles of polymerase chain reaction with the SureSelect forward and SureSelect ILM Pre-Capture Indexing reverse (Agilent) primers. The concentration and size distribution of the libraries were determined on an Agilent Bioanalyzer DNA 1000 chip.

Whole-exon capture was carried out by use of the protocol for the Agilent SureSelectXT Human All Exon V5+UTR kit. Briefly, 750 ng of the prepared library was incubated with whole-exon biotinylated RNA capture baits supplied in the kit for 24 hours at 65°C. The captured DNA:RNA hybrids were recovered with the use of Dynabeads MyOne Streptavidin T1 (Dynal). The DNA was eluted from the beads and purified with Ampure XP beads (Agencourt). The purified capture products were then amplified with the SureSelect Post-Capture Indexing forward and Index polymerase chain reaction reverse primers (Agilent) for 12 cycles.

Libraries were pooled at equimolar concentrations and loaded onto paired-end flow cells at concentrations of 7 to 8 pmol/L to generate cluster densities of 600,000 to 800,000 per 1 mm² following Illumina’s standard protocol with the use of the Illumina cBot and HiSeq paired-end cluster kit version 3. Each lane of a HiSeq flow cell produced 21 to 39 Gbases of sequence. The level of sample pooling was controlled by the size of the capture region and the desired depth of coverage.

The flow cells were sequenced as 101 × 2 paired-end reads on an Illumina HiSeq 2000 with TruSeq SBS sequencing kit version 3 and HiSeq data collection version 2.0.12.0 software. Base calling was performed with the Illumina RTA version 1.17.21.3. The Illumina paired-end reads were aligned to the hg19 reference genome through the use of Novoalign (http://novocraft.com) followed by the sorting and marking of duplicate reads with Picard (http://broadinstitute.github.io/picard/). Local realignment of insertion/deletions and base quality score recalibration were then performed with the Genome Analysis Toolkit.3 Single-nucleotide variants and insertions/deletions were called across all of the samples simultaneously using the Genome Analysis Toolkit Unified Genotyper with variant quality score recalibration.8

After WES, single-nucleotide variants and insertion/deletions were filtered to identify variants that followed either a sporadic or a recessive inheritance pattern with the use of Ingenuity Variant Software (Qiagen, Redwood City, CA). All variants were first filtered for a call quality score ≥20 and present in genes outside the top 1% of genes with high variability. To be considered a candidate mutation, the variant identified in the child had to be nonsynonymous (ie, amino acid altering: missense, nonsense, splice error, frame-shift insertion/deletions, or in-frame insertion/deletions). For the sporadic model, only ultrarare variants (allele frequency ≤0.01% in the 1000 Genome Project [1KG; n=1094 subjects: 381 whites, 246 blacks, 286 Asians, and 181 Hispanics9] and the National Heart, Lung and Blood Institute Grand Opportunity Exome Sequencing Project [NHBLI GO ESP]: n=6503 subjects: 4300 whites and 2203 blacks10] databases) that were absent in the exomes of both parents were considered. For the recessive inheritance model, only rare (allele frequency ≤1% in the 1KG and NHBLI GO ESP databases) variants present as either homozygotes (same mutation inherited from each parent) or compound heterozygotes (2 unique mutations in the same gene each inherited from a different parent) were considered.

Candidate gene priority ranking was performed with the use of 2 publicly available Web-based tools, Endevour (www.homes.esat.kuleuven.be/~biostu/endevour/tool/endevourweb.php) and ToppGene (www.toppgene.uchmc.org). The 3 most prevalent LQTS-causing genes (KCQ1, KCNH2, and SCN5A) were used to train these algorithms. Gene priority ranking was performed with default settings for each tool. The gene priority rankings from all tools were combined, and a final composite ranking was created.

DNA Sanger Sequencing for Variant Confirmation

Standard DNA dye terminator cycle sequencing protocols and an ABI Prism 377 automated sequencer (Applied Biosystems Inc, Foster City, CA) were used for Sanger sequencing confirmation of the TRDN mutation (c.del 53,56 ACAG). DNA sequence chromatograms were analyzed with Chromas version 1.45 (Queensland, Australia).

TRDN Mutational Analysis in Unrelated Phenotype-Positive/Genotype-Negative LQTS Patient Cohort

Thirty-three unrelated phenotype-positive/genotype-negative patients with a high-probability diagnosis of LQTS (QTc ≥500 milliseconds or an LQTS diagnostic score12 ≥3.5) were analyzed for TRDN mutations. All patients signed a Mayo Clinic Institutional Review Board–approved written consent form before genetic analysis.

Comprehensive mutational analysis of all 8 coding region exons of the TRDN gene (NM_001256021.1) that encodes the cardiac-specific isoform of triadin (also known as Trisk 32, NP_001242950) was performed on genomic DNA from these 33 patients with LQTS using polymerase chain reaction, denaturing high-performance liquid chromatography, and DNA Sanger sequencing. Only rare nonsynonymous homozygous or compound heterozygous mutations with a heterozygote frequency of ≤1% in the 1KG10 and the NHBLI GO ESP11 databases were considered pathogenic.

Statistical Analysis

A Fisher exact test (http://www.langsrud.com/fisher.htm) using a 2-tailed P value was used to compare the overall yield of TRDN mutations in patients ≤10 years of age with the yield observed in patients >10 years of age. This analysis was performed for the discovery subject (n=1) and the unrelated subjects (n=33).
Results

WES and Variant Filtration for the Identification of a Novel Pathogenic Substrate in a Sporadic/Recessive LQTS Pedigree

We performed WES analysis on a black family with presumed sporadic or autosomal-recessive LQTS after negative commercially available genetic testing for LQTS. The index case was a 10-year-old black girl who experienced syncope first at 1 year of age while riding her tricycle and then again at 2 years of age while dancing. She experienced cardiac arrest at 3 years of age. She was treated with propranol and an implantable cardioverter-defibrillator (ICD) and underwent left cardiac sympathetic denervation (LCSD) surgery. However, she continues to experience ventricular tachycardia (VT)– or ventricular fibrillation (VF)–terminating ICD shocks during exercise, emotional stimulation, and sleep. Her ECG provided evidence of a QTc of 500 milliseconds, along with extensive T-wave inversion in the precordial leads from V1 through V4 (Figure 1).

Although her LQTS diagnostic score was 7, consistent with high-probability LQTS, her stress test was atypical for LQTS with ventricular ectopy during the stress test and throughout the recovery phase until her heart rate was <85 bpm. Although her T-wave inversions satisfied one of the 2010 task force minor criteria for arrhythmogenic right ventricular cardiomyopathy (alternatively referred to as arrhythmogenic cardiomyopathy), she does not exhibit any major task force criteria for a clinical diagnosis of arrhythmogenic right ventricular cardiomyopathy. Specifically, both her echocardiogram and her cardiac computed tomography scan were negative for arrhythmogenic right ventricular cardiomyopathy. Both parents were considered unaffected, with normal ECGs and negative personal and family histories of cardiac-related events.

WES was performed on the affected index case and her unaffected parents. The subsequent WES mutational results were filtered, considering either a sporadic or an autosomal-recessive inheritance pattern (Figure 1). After WES, 123201

Figure 1. Whole-exome sequencing (WES) and familial genomic triangulation for the elucidation of a novel genetic substrate for long-QT syndrome (LQTS). A, The LQTS pedigree with a presumed sporadic/autosomal-recessive inheritance pattern showing the case–parent trio (yellow triangle), the affected index case (black circle), and the unaffected family members (white symbols). B, An ECG for the index case revealing a prolonged QTc and extensive T-wave inversion. C, A flow diagram of the variant filtering process and results for both a sporadic and a recessive inheritance model. D, Sanger sequencing confirmation of the TRDN mutation. The yellow triangle with a white square (unaffected father), white circle (unaffected mother), and black circle (affected index case) represents the case–parent trio who underwent WES. The (+) symbol represents a mutated allele, and the (−) symbol represents a normal allele. Shown are Sanger sequencing chromatograms from a normal control, the index case, and both parents. The underlined sequence (ACAG) represents the 4 deleted nucleotides that result in the p.D18fs*13 frameshift mutation. The location of the mutation is depicted on a linear topology of triadin.
total variants were identified within the 3 exomes. Of these, 116276 variants had a call quality score ≥20 and were within genes outside of the top 1% of exonically variable genes as indicated by Ingenuity Variant Software using data from the 1KG and NHLBI GO ESP databases. To be conservative in our sporadic approach, we limited the 116276 variants to only ultrarare variants with a frequency ≤0.01% in the 1KG and NHLBI GO ESP databases. This resulted in 8721 variants. These 942 represented nonsynonymous variants. Parental exon subtraction from the exome of the index case yielded 19 different genes.

For our recessive model, we first limited the 116276 variants to include only rare variants with a frequency ≤1% in the 1KG and NHLBI GO ESP databases. This resulted in 15726 variants. Of these, a total of 2273 variants were nonsynonymous. After index case–parent exon subtraction/inclusion criteria fitting a recessive inheritance pattern, there were 18 compound heterozygous variants in 9 genes and 5 autosomal homozygous recessive variants in 5 genes that remained.

Therefore, a total of 32 potential candidate genes (Table 1) with variants fitting either a sporadic (19 genes) or a recessive (14 genes) model were identified. One gene fit both models. Candidate gene priority ranking of the 32 genes with ToppGene and Endeavour identified TRDN, a gene that encodes for triadin (also known as Trisk 32) that critically regulates the cardiac muscle couplon structure and microdomain Ca²⁺ signaling in the heart, as the top-ranked LQTS-susceptibility gene.

Sanger DNA sequencing confirmed the presence of a homozygous TRDN deletion (c.del 53_56 ACAG) resulting in a frameshift mutation (p.D18fs*13) in the affected child (Figure 1). Both unaffected parents were heterozygous for the mutation. Notably, 1 black among the 1861 black exomes of the NHLBI GO ESP was a D18fs*13 carrier (0.054% carrier frequency). D18fs*13 was absent in the NHLBI GO ESP white exomes and the 1KG. In addition, D18fs*13 was reported as a heterozygote variant in 7 of 6185 individuals (0.01%) from the Exome Aggregation Consortium (ExAC) database overall and specifically in 7 of 4986 individual black exomes (0.14%).

**Spectrum and Prevalence of TRDN Mutations in a Cohort of Unrelated LQTS Patients**

Subsequently, 33 unrelated phenotype-positive/genotype-negative patients (Table 2) with a high-probability diagnosis of LQTS (QTc ≤500 milliseconds or an LQTS diagnostic score ≥3.5) were analyzed for TRDN mutations. Of these 33 cases, 21 were female and 12 were male. The average age at diagnosis was 23.6±18.7 years and ranged from 1 to 65 years. Nine patients were diagnosed at ≤10 years of age, and 24 were diagnosed at >10 years of age. The average QTc was 515±56 milliseconds. Forty-two percent of the patients had experienced syncope; 33% had cardiac arrest; and 36% had a positive family history of cardiac events. All 33 patients with LQTS were mutation negative for all 16 LQTS-associated genes identified to date, including (in alphabetic order) AKAP9, ANKB, CACNA1C, CALM1, CALM2, CALM3, CAV3, KCNE1, KCNE2, KCNH2, KCNJ2, KCNJ5, KCNQ1, SCN4B, SCN5A, and SNTA1.

After mutational analysis of the most prominent cardiac isoform of TRDN, 4 of 33 unrelated patients with LQTS (12.1%) had either homozygous or compound heterozygous TRDN mutations, including 3 unrelated patients with the same homozygous frameshift deletion (c.del 572_576 TAAGA) resulting in an immediate stop codon (p.K147fs*0). This mutation was absent in the NHLBI GO ESP, 1KG, and ExAC databases.

Interestingly, all 3 patients displayed a phenotype very similar to that of the patient with the homozygous p.D18fs*13 mutation identified by WES. The homozygous p.K147fs*0 mutation was identified in a 2-year-old Indian girl with an LQTS diagnostic score of 7.5 secondary to a QTc of 490 milliseconds, evidence of torsades de pointes, a personal history of syncope with stress, and a family history of unexplained
cardiac death (Figure 2 and Table 3). Electrocardiographically, she also exhibited persistent and extensive T-wave inversion in precordial leads V1 through V4 (Figure 3). She first experienced exercise-induced syncope at 1 year of age and then experienced SCA at 2 years of age. She received an ICD, underwent LCSD surgery, and was continued on β-blocker therapy. Subsequently, she experienced syncope while swimming and during dance class. At 6 years of age, she was noted to have slight proximal muscle weakness.

Despite both of her parents being considered unaffected with a normal QTc, there is a family history of a paternal first-degree cousin who experienced an unexplained sudden cardiac death at 6 years of age. Her parents’ genomic DNA was analyzed, with both parents having the same heterozygous p.K147fs*0 mutation, indicating an autosomal-recessive model of inheritance. Furthermore, her paternal uncle, the father of the 6-year-old cousin who experienced a sudden cardiac death, was also found to be heterozygous for p.K147fs*0. DNA was unavailable for the deceased cousin and her mother.

The same homozygous p.K147fs*0 mutation was also identified in a 5-year-old Arabic boy with an LQTS diagnostic score of 5.5 resulting from a QTc of 480 milliseconds, a personal history of syncope with stress, and a family history of unexplained cardiac arrest (Figure 2 and Table 3). On ECG, he also had extensive T-wave inversion in precordial leads V1 through V4 (Figure 3). He experienced exercise-induced syncope at 2 years of age, and his first cardiac arrest occurred at 2.5 years of age, followed by 2 more cardiac arrest events, which occurred within a month of one another. At 3 years of age, he underwent videoscopic LCSD surgery, was implanted with an ICD, and was maintained on β-blocker. After this treatment, he continued to experience several appropriate VF/VT–terminating ICD shocks. The patient’s parents are consanguineous first-degree cousins and both are phenotype-negative for LQTS. However, the patient’s older sister experienced syncope and cardiac arrest at 2 years of age. DNA was unavailable for the unaffected parents and the affected sibling.

The third unrelated patient with this same homozygous K147fs*0 frameshift mutation was a 6-year-old Indian girl with an LQTS diagnostic score of 7 (Figure 2 and Table 3). The family history was negative for cardiac events. The patient also had extensive T-wave inversion in precordial leads V1 through V4 and a QTc of 464 milliseconds (Figure 3). She first experienced syncope while swimming and during dance class. At 6 years of age, she was noted to have slight proximal muscle weakness.

The patient’s parents, who are consanguineous second cousins and phenotype-negative for LQTS, were heterozygous for the K147fs*0 mutation. The patient experienced cardiac arrest at 23 months and was defibrillated twice on scene from reported VF. The patient exhibited transient QT prolongation (QTc >500 milliseconds), and telemetry captured brief runs of polymorphic VT, including bidirectional VT. The latter arrhythmia prompted initial consideration of catecholaminergic polymorphic VT (CPVT). Akin to the previous cases, her ECG consistently and persistently exhibits T-wave inversions in leads III and IV and in the precordial leads V1 through V4 (Figure 3). After her cardiac arrest, she was dismissed with a combination therapy of β-blocker and ICD. She subsequently experienced 3 appropriate exertion-induced VF-terminating shocks from her ICD while on β-blocker therapy. Ultimately, she underwent videoscopic LCSD surgery and dismissed on once-a-day (10 mg) nadolol. However, she experienced her fourth appropriate shock after denervation, and her nadolol dose was increased to 10 mg twice daily.

Finally, a 2-year-old white male patient diagnosed with LQTS after a cardiac arrest at 20 months of age was compound heterozygous with 2 putative pathogenic TRDN mutations (Figure 2 and Table 3). The child had in utero bradycardia and ECGs documenting borderline QT intervals. After the child’s sentinel event of cardiac arrest with a documented VF at 20 months of age, he was diagnosed with possible LQTS. He was placed on β-blocker and implanted with an ICD as secondary prevention. He remained event free for a year until he experienced his first appropriate VF-terminating single ICD shock during exertion. Electrocardiographically, he presents with deeply inverted T waves in the precordial leads V1 through V4 and a QTc of 464 milliseconds (Figure 3). In addition, this child is now 4 years old and has begun exhibiting mild to moderate skeletal muscle weakness and decreased muscle tone.

This child was heterozygous for the same K147fs*0 frameshift mutation (maternally derived) and heterozygous for a paternally inherited putative splicing error mutation (c.22+29 A>G). The parents are both phenotypically normal with a negative family history of cardiac events. According to ESEfinder,1,16 a publically available online splice site prediction algorithm, the intron 1 wild-type donor splice site c.22+1_2 (GCTGAAGgtattgctacc) registers a weak splice site score (5.01380) below the default threshold of 6.67 of the algorithm for predicting a donor splice site, suggesting that this exon/intron 1 may be susceptible to alternative splicing. In fact, ESEfinder predicts that the alternative GT site at c.22+30_31 (gtataagagaaaa) actually has a higher score (6.18470) than the wild-type donor splice site but still below the default threshold. However, when altering the intronic nucleotide c.22+29 A to G, the alternative GT site (gtataagagaaaa) now scores 9.57210, well above the 6.67 threshold score for predicting this donor splice site. This strongly suggests that the alternative donor site would be preferentially used over the wild-type site, thus resulting in the inclusion of 29 additional nucleotides (c.22_23insGTATTGCTACCATTTTCCGATAGTATCAA) to exon 1, ultimately resulting in the translation of a p.N9fs*5 frameshift mutation. Furthermore, an in vitro minigene assay recently demonstrated that c.22+29 A>G produced abnormal splicing.17

### Table 2. Demographics of the Phenotype-Positive/Genotype-Negative LQTS Cohort

<table>
<thead>
<tr>
<th></th>
<th>LQTS (n=33)</th>
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<tbody>
<tr>
<td>Male/female</td>
<td>12/21</td>
</tr>
<tr>
<td>Average±SD age at diagnosis, y</td>
<td>23.6±18.7</td>
</tr>
<tr>
<td>Age range, y</td>
<td>1–65</td>
</tr>
<tr>
<td>Patients diagnosed at ≤10 y of age, n</td>
<td>9</td>
</tr>
<tr>
<td>Patients diagnosed at &gt;10 y of age, n</td>
<td>24</td>
</tr>
<tr>
<td>Average±SD QTc, ms</td>
<td>515±56</td>
</tr>
<tr>
<td>Patients with syncope, %</td>
<td>42</td>
</tr>
<tr>
<td>Patients with cardiac arrest, %</td>
<td>33</td>
</tr>
<tr>
<td>Patients with a positive family history, %</td>
<td>36</td>
</tr>
</tbody>
</table>

LQTS indicates long-QT syndrome.
This c.22+29 A>G mutation is absent in the NHLBI GO ESP (n=6503 subjects) but was present in 1 of 60698 individual exomes of the ExAC database.\textsuperscript{15}

With the inclusion of the original patient identified by WES, 5 of 34 of the genetically elusive LQTS cohort (14.7%) had homozygous or compound heterozygous frameshift/
premature truncating mutations in TRDN. Remarkably, all 5 patients have exhibited a similar collective phenotype: autosomal-recessive LQTS with normal hearing; QT prolongation with extensive (V1–V4) precordial lead T-wave inversions; exertion-triggered cardiac events, including cardiac arrest before 3 years of age; continued cardiac events refractory to both β-blockers and LCSD surgery; and potentially a noncardiac phenotype of skeletal muscle weakness. Moreover, none of the 30 TRDN mutation–negative patients with LQTS displayed these clearly defined phenotypic features. Notably, all 5 individuals with either TRDN homozygous or compound heterozygous frameshift mutations experienced cardiac arrest at a very early age (≤3 years of age). In fact, the yield of TRDN mutations was significantly higher among the subset diagnosed at ≤10 years of age (5 of 10, 50%) compared with those patients who were >10 years of age (0 of 24, 0%; P<0.001; Figure 4).

Here, we identified TRDN-encoded triadin as a novel genetic basis for recessively inherited LQTS. In fact, nearly 15% of our overall and 50% of our childhood-aged (≤10 years) genetically elusive LQTS cohort hosted homozygous/compound heterozygous TRDN frameshift mutations. However, because this is a small cohort, the prevalence may not be reflective of the general population of gene-negative LQTS. Because frameshift mutations often result in nonfunctional proteins or immediate nonsense-mediated RNA decay, these patients are most likely triadin null.

TRDN, a gene expressed in both cardiac and skeletal muscle, undergoes extensive alternative splicing to produce several isoforms.21–23 Cardiac triadin is a 286–amino acid transmembrane protein with a short 47–amino acid N-terminal cytoplasmic region, amino acids 48 to 68 imbedded in the junctional sarcoplasmic reticulum membrane, and a long, positively charged C-terminal tail extending into the sarcoplasmic reticulum lumen.24 The first 264 amino acids of both the cardiac and skeletal muscle triadin isoforms are encoded by the same first 8 exons of the 41-exon TRDN gene. Therefore, mutations within exons 1 through 8 of the TRDN gene could possibly affect the function of both the cardiac and skeletal muscle isoforms of TRDN.

Cardiac triadin is critical to the structure and functional regulation of cardiac muscle calcium release units and excitation-contraction coupling. Structural or functional disruption of this cardiac calcium release unit can lead to significant ventricular arrhythmias. In fact, ablation of cardiac triadin

**Discussion**

WES, coupled with familial triangulation and systems biology/disease-network analysis–based gene ranking, has become a useful technique to identify novel genetic mechanisms of disease, specifically LQTS. Recently, we used this strategy to identify a gain-of-function mutation in the CACNA1C-encoded L-type calcium channel (LTCC) as responsible for autosomal-dominant LQTS.18 Similarly, a child–parent trio WES-based approach was used to identify mutations in calmodulin that were responsible for sporadic LQTS.19,20

![Figure 3. Representative ECG for patients 2 through 5. ECGs display the common ECG signature of extensive T-wave inversion in precordial leads V1 through V4 with QT prolongation.](http://circ.ahajournals.org/)

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causes loss of cardiac calcium release units, impaired excitation-contraction coupling, and cardiac arrhythmias in TRDN-null mice, particularly during β-adrenergic stimulation. The structural remodeling of the dyad, together with cardiomyocyte Ca\(^{2+}\) overload as a result of slower Ca\(^{2+}\)-dependent inactivation of the LTCC, precipitated the stress-induced VT in the TRDN-null mice. Therefore, it is biologically plausible to hypothesize that the first 264 amino acids encoded by the TRDN gene are essential for the antiarrhythmic function of triadin, as this region of the protein is proximal to the structural scaffold that anchors the dyad.28

Slower LTCC inactivation could lengthen the cardiac action potential and manifest as a prolonged QT interval on ECG. In fact, CACNA1C-mediated LQTS results from a gain-of-function in the LTCC channel through either increased peak I\(_{Ca,L}\) or slower channel inactivation. Furthermore, LQTS-causing calmodulin mutations disrupt calcium-dependent inactivation of the LTCC. Therefore, it is biologically plausible that a decrease in I\(_{Ca,L}\) inactivation, caused by loss of triadin, could lead to prolonged cardiac action potential and an LQTS phenotype.

Interestingly, TRDN mutations have been implicated previously in recessively inherited CPVT, being identified in 2 of 97 patients diagnosed with CPVT (2%) who were genotype-negative for RYR2 or CASQ2 mutations. Specifically, a homozygous p.D18fs*13 mutation was identified in a 2-year-old boy who experienced exertion-induced syncope and cardiac arrest, and compound heterozygous TRDN mutations (p.T59R and p.Q205X) were identified in a 26-year-old man who experienced recurrent exertion-induced syncope since infancy. In both cases, the patients’ resting ECGs were stated to be normal with no QT-interval prolongation.

Curiously, we identified this precise TRDN homozygous p.D18fs*13 mutation in a patient with CPVT with a QTc of 500 milliseconds and p.K147fs*0 in 4 additional unrelated patients with LQTS with QT prolongation. Although our patients and the patients with CPVT reported by Roux-Buisson et al27 are phenotypically similar, our findings suggest that homozygous/compound heterozygous TRDN mutations may underlie malignant, autosomal-recessive LQTS. We have examined 42 unrelated patients with genetically elusive CPVT/idiopathic VF, and no TRDN mutations were identified (data not shown).

Remarkably, all 5 TRDN-null patients displayed the common ECG phenotype of extensive T-wave inversions in precordial leads V\(_1\) through V\(_6\), severe disease expression of exercise-induced cardiac arrest in early childhood (≤3 years of age), and a recessive inheritance pattern and required aggressive therapy. Despite combination therapy with β-blockers and LCSD, recurrent LQTS-triggered events, including appropriate VF-terminating ICD shocks, have occurred. Considering that the first 264 amino acids encoded by the TRDN gene are present in both the cardiac and skeletal muscle triadin isoforms, one might also anticipate an emerging skeletal muscle phenotype as already exhibited by 2 of the children in this study and 1 of the patients described by Roux-Buisson et al.27 In addition, triadin-null mice present with muscle weakness, possibly resulting from the reduction in releasable calcium observed in primary myotubes after ryanodine receptor stimulation.

Although all 5 cases with a strikingly similar phenotype host either homozygous (D18fs*13 or K147fs*0; 4 cases) or compound heterozygous (K147fs*0 and p.N9fs*5; 1 case) TRDN frameshift mutations that would result in early truncation of the triadin protein and presumably result in each patient being essentially TRDN null, we have not functionally characterized each specific mutation, which is a potential study limitation. Although the creation and study of TRDN-mutation specific mice or the use of patient-specific induced pluripotent stem cell cardiomyocytes would be desirable, these studies are beyond the scope of this work. However, previous studies on TRDN-null mice that were engineered through targeted removal of the first exon of the TRDN gene provide substantial support for the proposed mechanism of disease phenotype observed in our TRDN-null patients.28

On the basis of the TRDN-null mice observations, an LTCC channel blocker such as nifedipine or verapamil may be a therapeutic consideration for these patients. Given these atypical LQTS phenotypic features that are in common among TRDN-null patients, we propose that either triadin knockout syndrome (Table 4) or TRDN-mediated autosomal-recessive LQTS should be used rather than LQT17. In fact, the mechanistic insights observed in the TRDN-null mice support this as a unique disorder. Unlike the mechanism of sarcomplasmic reticulum Ca\(^{2+}\) leak and triggered beats at normal or decreased sarcomplasmic reticulum Ca\(^{2+}\) load observed with ryanodine receptor 2/calsequestrin 2-mediated CPVT, loss of cardiac triadin dramatically reduces the negative feedback on the LTCC, resulting in a net increase in Ca\(^{2+}\) influx into the cell and leading to myocyte Ca\(^{2+}\) overload, increases in spontaneous sarcomplasmic reticulum Ca\(^{2+}\) release frequency, and VT, particularly in the setting of β-adrenergic stimulation.

Although the prevalence of triadin-truncating mutations in the general Middle Eastern or South Asian populations is not currently known, because 3 of our 5 cases descended from these geographical regions and all were found to be homozygous for the K147fs*0 mutation, we suspect that the incidence of triadin knockout syndrome could be even greater in this region of the world where consanguineous marriages may constitute 20% to 50% of all marriages.29 Provided the potential devastating outcome of pediatric sudden cardiac death associated with triadin knockout syndrome, the phenotypic

![Figure 4](http://circ.ahajournals.org/)

**Figure 4.** The effect of age on the percent containing a TRDN mutation. The graph compares the percent containing a TRDN mutation identified in patients ≤10 years of age vs patients >10 years.

<table>
<thead>
<tr>
<th>Age</th>
<th>Percent Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 10 years</td>
<td>50%</td>
</tr>
<tr>
<td>&gt; 10 years</td>
<td>0%</td>
</tr>
</tbody>
</table>

\(P < 0.001\)
and genotypic characterization of this newly described syndrome may have profound implications for prematernal and preconception genetic counseling, especially in highly consanguineous populations.

With the identification of recessively inherited TRDN frameshift mutations in early childhood LQTS patients manifesting with multiple episodes of exertion-induced syncope/cardiac arrest, a common ECG signature of extensive T-wave inversion in precordial leads V5 through V6, consistent with transient QT prolongation, and the possibility of skeletal muscle weakness, we describe for the first time triadin knockout syndrome. Given the recurrent nature of potential lethal arrhythmias, patients fitting this phenotypic profile should undergo cardiac TRDN genetic testing.

Acknowledgments

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Table 4. Summary of Triadin Knockout Syndrome

<table>
<thead>
<tr>
<th>Inheritance pattern</th>
<th>Autosomal recessive (homozygous or compound heterozygous TRDN mutations)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrocardiographically</td>
<td>Extensive T-wave inversions in precordial leads V5-V6</td>
</tr>
<tr>
<td>Consistent or transient QT prolongation</td>
<td></td>
</tr>
<tr>
<td>Phenotypic expression</td>
<td>Severe disease expression of exercise-induced syncope/cardiac arrest in early childhood (≤5 y), often with first presentation during the second year of life</td>
</tr>
<tr>
<td>Possible noncardiac involvement with mild to moderate skeletal muscle weakness</td>
<td></td>
</tr>
<tr>
<td>May require aggressive therapy</td>
<td></td>
</tr>
<tr>
<td>β-Blocker*</td>
<td></td>
</tr>
<tr>
<td>Implantation of an ICD</td>
<td></td>
</tr>
<tr>
<td>LCSD surgery</td>
<td></td>
</tr>
</tbody>
</table>

*ICD indicates implantable cardioverter-defibrillator; and LCSD, left cardiac sympathetic denervation.

References


Long-QT syndrome (LQTS) is a genetic disorder of myocardial repolarization that may result in syncope, seizures, or sudden cardiac arrest. Although 16 LQTS-susceptibility genes have been discovered, 20% of LQTS remains genetically elusive. Using whole-exome sequencing child–parent trio analysis followed by recessive and sporadic inheritance modeling and disease-network candidate analysis gene ranking, we discovered a novel underlying genetic mechanism for recessively inherited LQTS. A homozygous p.D18fs*13 TRDN-encoded triadin frameshift mutation was discovered in a 10-year-old female LQTS patient with a QTc of 500 milliseconds who experienced recurrent exertion-induced syncope/cardiac arrest beginning at 1 year of age. Subsequent mutational analysis of TRDN revealed either homozygous or compound heterozygous frameshift mutations in 4 of 33 unrelated cases of LQTS (12%). All 5 TRDN-null patients displayed extensive T-wave inversions in precordial leads V_1 through V_4, with either persistent or transient QT prolongation and severe disease expression of exercise-induced cardiac arrest in early childhood (≤3 years of age) and required aggressive therapy. We identified TRDN as a novel underlying genetic basis for recessively inherited LQTS. All TRDN-null patients had a strikingly similar phenotype that has been called triadin knockout syndrome. Given the recurrent nature of potential lethal arrhythmias, patients fitting this phenotypic profile should undergo cardiac TRDN genetic testing.
Homozygous/Compound Heterozygous Triadin Mutations Associated With Autosomal-Recessive Long-QT Syndrome and Pediatric Sudden Cardiac Arrest: Elucidation of the Triadin Knockout Syndrome
Helene M. Altmann, David J. Tester, Melissa L. Will, Sumit Middha, Jared M. Evans, Bruce W. Eckloff and Michael J. Ackerman

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