Homozygous/Compound Heterozygous Triadin Mutations Associated with Autosomal Recessive Long QT Syndrome and Pediatric Sudden Cardiac Arrest: Elucidation of Triadin Knockout Syndrome

Running title: Altmann et al.; Triadin Knockout (TKO) Syndrome

Helene M. Altmann¹*; David J. Tester, BS¹,2*; Melissa L. Will, BS¹,2; Sumit Middha, MS³; Jared M. Evans, MS³; Bruce W. Eckloff, BS⁴; Michael J. Ackerman, MD, PhD¹,2,5

¹Dept of Molecular Pharmacology & Experimental Therapeutics; Windland Smith Rice Sudden Death Genomics Laboratory, Mayo Clinic, Rochester, MN; ²Dept of Medicine/Division of Cardiovascular Diseases, Mayo Clinic, Rochester, MN; ³Division of Biomedical Statistics and Informatics, Dept of Health Sciences Research, Mayo College of Medicine, Rochester, MN; ⁴Medical Genome Facility, Mayo Clinic, Rochester, MN; ⁵Dept of Pediatrics/Division of Pediatric Cardiology, Mayo Clinic, Rochester, MN

*contributed equally

Address for Correspondence:
Michael J. Ackerman, MD, PhD
Mayo Clinic Windland Smith Rice Sudden Death Genomics Laboratory
Guggenheim 501, Mayo Clinic
200 First Street SW
Rochester, MN 55905
Tel: 507-284-0101
Fax: 507-284-3757
E-mail: ackerman.michael@mayo.edu

Journal Subject Codes: Etiology:[109] Clinical genetics, Etiology:[5] Arrhythmias, clinical electrophysiology, drugs
Abstract

Background—LQTS may result in syncope, seizures, or sudden cardiac arrest. Although 17 LQTS-susceptibly genes have been discovered, 20-25% of LQTS remains genetically elusive. Methods and Results—Whole exome sequencing (WES) child-parent trio analysis followed by recessive and sporadic inheritance modelling and disease-network candidate analysis gene ranking was performed to identify a novel underlying genetic mechanism for LQTS. Subsequent mutational analysis of the candidate gene was performed using PCR, DHPLC, and DNA sequencing on a cohort of 33 additional unrelated patients with genetically elusive LQTS. Following WES and variant filtration, a homozygous p.D18fs*13 TRDN-encoded triadin frame-shift mutation was discovered in a 10 year-old female LQTS patient with a QTc of 500 ms who experienced recurrent exertion-induced syncope/cardiac arrest beginning at age 1 year. Subsequent mutational analysis of TRDN revealed either homozygous or compound heterozygous frame-shift mutations in 4/33 (12%) unrelated cases of LQTS. All five TRDN null patients displayed extensive T-wave inversions in precordial leads V1-V4, with either persistent or transient QT-prolongation, 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 (5/10, 50%) compared to older patients (0/24, 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: long QT syndrome, arrhythmia (heart rhythm disorders), genetics, human, pediatrics, heart arrest
Sudden cardiac death is a major world-wide public health burden with an estimated annual incidence ranging from 180,000 to 450,000 in the United States (US) 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 one year of age and another 2000-5000 young people between 1 and 35 years die following SCA yearly. Those who survive a SCA are often left with neurological sequelae.

Long QT Syndrome (LQTS), with an estimated prevalence of 1:2500, 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 electrocardiogram, may manifest as syncope, seizures, or SCA typically following a precipitating event such as exertion, extreme emotion, auditory stimuli, or even at rest. Identifying and understanding the genetic etiology can have a profound life-saving impact on the overall clinical management and prophylactic treatment of an LQTS patient.

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 due to mutations within three genes (KCNQ1, KCNH2, and SCN5A) that encode for critical ion channel alpha-subunits responsible for the cardiac action potential. However, ~20% of patients with clinically definite LQTS remain genetically elusive.

In this study, we performed whole exome sequencing (WES) to identify a novel genetic explanation for a 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 LQTS patients. Collectively, the phenotype for this novel,
potentially lethal syndrome, termed triadin knockout (TKO) syndrome, is detailed.

**Methods**

**Sporadic/Autosomal Recessive LQTS Pedigree**

An African American 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 following negative commercially available genetic testing for LQTS. The index case was a 10-year-old female 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 ECGs and a negative personal and family history for cardiac related events. Following written informed consent for this Mayo Clinic IRB approved study; blood was collected for all three family members and genomic DNA was isolated.

**Whole Exome Sequencing (WES)**

WES and subsequent variant annotation was performed on genomic DNA derived from the symptomatic 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) using the Bravo liquid handler from Agilent. Briefly, 1-3 ug of genomic DNA was fragmentated to 150-200 bp using 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’ end were ligated and the resulting constructs were purified using AMPure SPRI beads (Agencourt). The adapter-modified DNA fragments were enriched by 4 cycles of PCR using SureSelect forward and SureSelect ILM Pre-Capture Indexing reverse (Agilent) primers. The concentration and size distribution of the
libraries was determined on an Agilent Bioanalyzer DNA 1000 chip.

Whole exon capture was carried out using the protocol for Agilent’s Sure SelectXT Human All Exon V5+UTR kit. Briefly, 750 ng of the prepped 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 using Dynabeads MyOne Streptavidin T1 (Dynal). The DNA was eluted from the beads and purified using Ampure XP beads (Agencourt). The purified capture products were then amplified using the SureSelect Post-Capture Indexing forward and Index PCR reverse primers (Agilent) for 12 cycles.

Libraries were pooled at equimolar concentrations and loaded onto paired end flow cells at concentrations of 7-8 pM to generate cluster densities of 600,000-800,000/mm² following Illumina’s standard protocol using the Illumina cBot and HiSeq Paired end cluster kit version 3. Each lane of a HiSeq flow cell produced 21-39 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 X 2 paired end reads on an Illumina HiSeq 2000 using TruSeq SBS sequencing kit version 3 and HiSeq data collection version 2.0.12.0 software. Base-calling was performed using Illumina’s RTA version 1.17.21.3.

The Illumina paired end reads were aligned to the hg19 reference genome using Novoalign (http://novocraft.com) followed by the sorting and marking of duplicate reads with Picard (http://picard.sourceforge.net). Local realignment of INDELs and base quality score recalibration were then performed using the Genome Analysis Toolkit (GATK)⁸. Single nucleotide variants (SNVs) and insertions/deletions (INDELs) were called across all of the samples simultaneously using GATK's UnifiedGenotyper with variant quality score recalibration⁹.
Following WES, single nucleotide variants (SNVs) and insertion/deletions (INDELs) were filtered to identify variants which followed either a sporadic or recessive inheritance pattern using 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 non-synonymous (i.e. amino acid altering: missense, nonsense, splice-error, frame-shift INDEL, or in-frame INDEL). For the sporadic model, only ultra-rare variants (allele frequency ≤ 0.01% in the 1,000 Genome Project [1KG, n=1094 subjects; 381 Caucasian, 246 African-American, 286 Asians, and 181 Hispanics]\(^\text{10}\) and the National Heart, Lung and Blood Institute Grand Opportunity [NHLBI GO ESP] Exome Sequencing Project [n=6503 subjects; 4300 Caucasians and 2203 African-Amerian]\(^\text{11}\) 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 the NHLBI GO ESP databases) variants present as either homozygotes (same mutation inherited from each parent) or compound heterozygotes (two unique mutations in the same gene each inherited from a different parent) were considered.

Candidate gene priority ranking was performed using two publicly available web-based tools, Endeavour ( www.homes.esat.kuleuven.be/~bioiuser/endeavour/tool/endeavourweb.php ) and ToppGene ( www.toppgene.cchmc.org ). The 3 most prevalent LQTS-causing genes (\(\text{KCNQ1}, \text{KCNH2},\) and \(\text{SCN5A}\)) were used to train these algorithms. Gene priority ranking was performed using default settings for each tool. The gene priority ranking from each tool was 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 using 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 ms or a LQTS diagnostic score ≥ 3.512), were analyzed for
TRDN mutations. All patients signed a Mayo Clinic IRB-approved written consent form prior to
genetic analysis.

Comprehensive mutational analysis of all 8 coding region exons of the TRDN gene
(NM_001256021.1) that encodes the cardiac-specific isoform triadin (also known as Trisk 32,
NP_001242950) was performed on genomic DNA from these 33 LQTS patients using PCR,
DHPLC, and DNA Sanger Sequencing. Only rare non-synonymous homozygous or compound
heterozygous mutations with a heterozygote frequency of ≤ 1% in the 1KG10 and the NHLBI GO
ESP11 were considered to be pathogenic.

**Statistical Analysis**

A Fisher’s Exact Test (http://www.langsrud.com/fisher.htm) employing a 2-tail p-value was used
when comparing the overall yield of TRDN mutations in patients that are ≤ 10 years of age to the
yield observed in patients that are > 10 years of age. This analysis was performed for the
discovery subject (n=1) plus the unrelated subjects (n=33).
Results

Whole Exome Sequencing (WES) and Variant Filtration for the Identification of a Novel Pathogenic Substrate in a Sporadic/Recessive LQTS Pedigree

We performed whole exome sequencing (WES) analysis on an African American family with presumed sporadic or autosomal recessive LQTS following negative commercially available genetic testing for LQTS. The index case was a 10-year-old African American female who first experienced syncope 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 propanol, 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 evidenced a QTc of 500 ms along with extensive T-wave inversion in the precordial leads from V1-V4 (Figure 1).

Although her LQTS diagnostic score\(^\text{12}\) 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 beats per minute. Although her T-wave inversions satisfied one of the 2010 task force minor criteria for arrhythmogenic right ventricular cardiomyopathy (ARVC, alternatively referred to as arrhythmogenic cardiomyopathy or ACM), she does not exhibit any major task force criteria for a clinical diagnosis of ARVC/ACM.\(^\text{13}\)

Specifically, both her echocardiogram and her cardiac computed tomography (CT) scan were negative for ARVC. Both parents were considered unaffected with normal ECGs and a negative personal and family history for 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 autosomal recessive inheritance pattern (Figure 1). Following WES, 123,201 total variants were identified within the three exomes. Of these, 116,276 variants had a call quality score ≥ 20 and 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\textsuperscript{10,11}. To be conservative in our sporadic approach, we limited the 116,276 variants to only ultra-rare variants with a frequency ≤ 0.01% in the 1KG and NHLBI GO ESP databases\textsuperscript{10,11}. This resulted in 8,721 variants. Of these, 942 represented non-synonymous variants. Parental exome subtraction from the index case’s exome yielded 26 sporadic variants (identified in the case and absent in either parent) in 19 different genes.

For our recessive model, we first limited the 116,276 variants to only include rare variants with a frequency ≤ 1% in the 1KG and NHLBI GO ESP databases\textsuperscript{10,11}. This resulted in 15,726 variants. Of these, a total of 2,273 variants were non-synonymous. Following index case-parent exome 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 (Table 1) potential candidate genes with variants fitting either a sporadic (19 genes) or recessive model (14 genes) were identified. One gene fit both models. Candidate gene priority ranking of the 32 genes using ToppGene and Endeavour identified \textit{TRDN}, a gene which encodes for triadin (also known as Trisk 32) that critically regulates the cardiac muscle couplon structure and microdomain Ca\textsuperscript{2+} signaling in the heart\textsuperscript{14}, as the top ranked LQTS-susceptibility gene.

Sanger DNA sequencing confirmed the presence of a homozygous \textit{TRDN} deletion (c.del
53_56 ACAG) resulting in a frame-shift mutation (p.D18fs*13) in the affected child (Figure 1). Both unaffected parents were heterozygous for the mutation. Notably, 1 African American among the 1861 African American exomes of the NHLBI GO ESP was a D18fs*13 carrier (0.054% carrier frequency). D18fs*13 was absent in the NHLBI GO ESP Caucasian exomes and the 1KG10,11. In addition, D18fs*13 was reported as a heterozygote variant in 7/61,085 (0.01%) individuals from the Exome Aggregation Consortium (ExAC) database overall and specifically, in 7 / 4986 (0.14%) individual African exomes15.

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 ms or a LQTS diagnostic score ≥ 3.512) 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 ms. 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 LQTS patients were mutation negative for all 16 LQTS-associated genes identified to date including (in alphabetical order), AKAP9, ANKB, CACNA1C, CALM1, CALM2, CALM3, CAV3, KCNE1, KCNE2, KCNH2, KCNJ2, KCNJ5, KCNQ1, SCN4B, SCN5A, and SNTA1.

Following mutational analysis of the most prominent cardiac isoform of TRDN, 4/33 (12.1%) unrelated LQTS patients had either homozygous or compound heterozygous TRDN mutations including three unrelated patients with the same homozygous frame-shift 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 databases10,11,15.
Interestingly, all three patients displayed a phenotype very similar to 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 female with a LQTS diagnostic score of 7.5 secondary to a QTc of 490 ms, evidence of torsades de pointes, personal history of syncope with stress, and a family history of unexplained cardiac death (Figure 2, Table 3). Electrocardiographically, she also exhibited persistent and extensive T-wave inversion in precordial leads V1 – V4 (Figure 3). She first experienced exercise-induced syncope at one year of age and then experienced SCA at 2 years of age. She received an ICD, underwent LCSD surgery, and was continued on beta-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 her parents both 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 (SCD) at the age of 6 years. 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 SCD, 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 male with a LQTS diagnostic score of 5.5 due to a QTc of 480 ms, personal history of syncope with stress, and a family history of unexplained cardiac arrest (Figure 2, Table 3). On ECG, he too had extensive T-wave inversion in precordial leads V1-V4 (Figure 3). He experienced exercise-induced syncope at two years of age and his first cardiac arrest occurred at 2.5 years of age, followed by two more cardiac arrest events, which occurred within a month of one another.
At age 3 years, he underwent videoscopic LCSD surgery, was implanted with an ICD, and was maintained on beta-blocker. Following this treatment strategy, he continued to experience several appropriate VF/VT-terminating ICD shocks. The patient’s parents are consanguineous first degree cousins and are both 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 frame-shift mutation, was a 6-year-old Indian female with a LQTS diagnostic score of 7 (Figure 2, Table 3). The family history was negative for cardiac events. The patient’s parents, who are consanguineous second cousins and both phenotype-negative for LQTS, were each 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 ms) and telemetry captured brief runs of polymorphic-VT including bidirectional-VT. The latter arrhythmia prompted initial consideration of catecholaminergic polymorphic ventricular tachycardia (CPVT). Akin to the previous cases, her ECG consistently and persistently exhibits T-wave inversions in leads III and IV as well as the precordial leads of V1-V4 (Figure 3).

Following her cardiac arrest, she was dismissed with a combination therapy of beta-blocker and ICD. She subsequently experienced 3 appropriate exertion-induced VF-terminating shocks from her ICD while on beta-blocker therapy. Ultimately, she underwent videoscopic LCSD surgery and dismissed on one-a-day (10 mg) nadolol. However, she experienced her fourth appropriate shock post-denervation and her nadolol dose was increased to 10 mg twice-a-day.

Finally, a 2-year-old Caucasian male patient diagnosed with LQTS following a cardiac arrest at age 20 months, was compound heterozygous with two putative pathogenic TRDN
mutations (Figure 2, Table 3). The child had in utero bradycardia and ECGs documenting borderline QT-intervals. Following the child’s sentinel event of cardiac arrest with documented VF at 20 months of age, he was diagnosed with possible LQTS. He was placed on beta-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-V4 and a QTc of 464 ms (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 frame-shift 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 ESE finder 16, a publically available on-line splice-site prediction algorithm, the intron 1 wild-type donor splice-site c.22+1_2 (GCTGAAGgtatctacc) registers a weak splice site score (5.01380) below the algorithm’s default threshold of 6.67 for predicting a donor splice-site, suggesting that this exon/intron 1 may be susceptible to alternative splicing. In fact, ESE finder predicts the alternative GT site at c.22+30_31 (gtatcaggtaagaaaa) 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 a G, the alternative GT site (gtatcaggtaagaaaa) now scores a 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_23insGTATTGCTACCATTTCGATAGTATCAAA) to exon 1, ultimately resulting in the translation of a p.N9fs*5 frame-shift mutation. Furthermore, an in vitro minigene
assay recently demonstrated that c.22+29 A>G produced abnormal splicing\textsuperscript{17}. This c.22+29 A>G mutation is absent in NHLBI GO ESP (n=6503 subjects) but was present in 1/60,698 individual exomes of the ExAC database\textsuperscript{15}.

Including the original patient identified by WES, 5/34 (14.7\%) of the genetically-elusive LQTS cohort had homozygous or compound heterozygous frame-shift/premature truncating mutations in \textit{TRDN}. Remarkably, all five 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 beta-blockers and LCSD surgery, and potentially a non-cardiac phenotype of skeletal muscle weakness. Moreover, none of the 30 TRDN mutation negative LQTS patients displayed these clearly defined phenotypic features. Notably, all 5 individuals with either \textit{TRDN} homozygous or compound heterozygous frame-shift mutations experienced cardiac arrest at a very early age (≤ 3 years of age). In fact, the yield of \textit{TRDN} mutations was significantly higher among the subset diagnosed ≤ 10 years of age (5/10, 50\%) compared to those patients older than 10 years of age (0/24, 0\%, p<0.001, \textit{Figure 4}).

\textbf{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 \textit{CACNA1C}-encoded L-type calcium channel (LTCC) as responsible for autosomal dominant LQTS\textsuperscript{18}. Similarly, a child-parent trio WES-based approach was used to identify mutations in
calmodulin that were responsible for sporadic LQTS\textsuperscript{19,20}.

Herein, 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 (\( \leq 10 \) years) genetically elusive LQTS cohort hosted homozygous/compound heterozygous TRDN frame-shift mutations. However since this is a small cohort, the prevalence may not be reflective of the general population of gene-negative LQTS. Since frame-shift mutations often result in non-functional proteins or immediate nonsense-mediated RNA decay, these patients are most likely triadin null.

\textit{TRDN} is a gene expressed in both cardiac and skeletal muscle and undergoes extensive alternative splicing in order to produce several isoforms\textsuperscript{21-23}. Cardiac triadin is a 286 amino acid transmembrane protein with a short 47 amino acid N-terminal cytoplasmic region, amino acids 48-68 imbedded in the junctional sarcoplasmic reticulum (jSR) membrane, and a long positively charged C-terminal tail extending into the SR lumen\textsuperscript{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 \textit{TRDN} gene. Therefore, mutations within exons 1-8 of \textit{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 (CRU) and excitation-contraction coupling. Structural or functional disruption of this cardiac CRU can lead to significant ventricular arrhythmias. In fact, ablation of cardiac triadin causes loss of cardiac CRUs, impaired excitation-contraction coupling, and cardiac arrhythmias in \textit{TRDN} null mice, particularly during $\beta$-adrenergic stimulation\textsuperscript{14}. 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 \textit{TRDN} null mice\textsuperscript{14}.
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 a LQTS phenotype.

Interestingly, TRDN mutations have been implicated previously in recessively inherited CPVT being identified in 2/97 (2%) patients diagnosed with CPVT 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 male who experienced recurrent exertion-induced syncope since infancy. In both cases, their 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 LQTS patient with a QTc of 500 ms and p.K147fs*0 in 4 additional unrelated LQTS patients. While our patients and the CPVT patients reported by Roux-Buission 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/IVF and no TRDN mutations were identified (data not shown).

Remarkably, all five TRDN null patients displayed the common electrocardiographic phenotype of extensive T-wave inversions in precordial leads V1-V4, severe disease expression of exercise-induced cardiac arrest in early childhood (≤ 3 years of age), a recessive inheritance
pattern, and required aggressive therapy. Despite combination therapy with beta-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 two of the children in this study as well as one of the patients described by Roux-Buission. In addition, triadin null mice present with muscle weakness possibly due to reduction of releasable calcium observed in primary myotubules following RyR 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 frame-shift 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 constituting a potential study limitation. While the creation and study of TRDN mutation specific mice or the use of patient specific induced pluripotent stem cell cardiomyocytes (iPS-CM) would be desirable, these studies are beyond the scope of this current manuscript. However, previous studies on TRDN null mice that were engineered through targeted removal of the first exon of the TRDN gene do provide substantial support in the proposed mechanism of disease phenotype observed in our TRDN null patients.

Based on the TRDN null mice observations, a LTCC channel blocker, like 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 (TKO) 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 SR Ca\(^{2+}\) leak and triggered beats at normal or decreased SR Ca\(^{2+}\) load observed with RYR2/CASQ2-mediated CPVT, loss of cardiac triadin dramatically reduces the negative feedback on the LTCC resulting in net increase in Ca\(^{2+}\) influx into the cell leading to myocyte Ca\(^{2+}\) overload, increases in SCR frequency, and VT, particularly in the setting of β-adrenergic stimulation.

While the prevalence of triadin truncating mutations in the general Middle Eastern or South Asian populations is not currently known, because 3/5 of our cases descended from these geographical regions and all were found to be homozygous for K147fs*0 mutation, we suspect the incidence of TKO syndrome could be even greater in this region of the world where consanguineous marriages may constitute 20-50% of all marriages. Provided the potential devastating outcome of pediatric sudden cardiac death associated with TKO syndrome, the phenotypic and genotypic characterization of this newly described syndrome may have profound implications for premarital and preconception genetic counseling, especially in highly consanguineous populations.

With the identification of recessively inherited \(TRDN\) frame-shift mutations in early childhood LQTS patients manifesting with multiple episodes of exertion-induced syncope/cardiac arrest, a common electrocardiographic signature of extensive T-wave inversion in precordial leads V1-V4 with consistent or transient QT-prolongation, and the possibility of skeletal muscle weakness, we describe for the first time tridain knockout (TKO) syndrome. Given the recurrent nature of potential lethal arrhythmias, patients fitting this phenotypic profile should undergo cardiac \(TRDN\) genetic testing.

Acknowledgments: We would like to acknowledge gratefully the physicians who referred these patients to our institution: Lee B. Beerman, MD, Children’s Hospital of Pittsburgh of UPMC,
Division of Pediatric Cardiology, Pittsburgh, PA; Peter S. Fischbach, MD, Emory University School of Medicine and Children’s Healthcare of Atlanta, Pediatric Cardiology, Atlanta, GA; Christopher S. Snyder, MD, University Hospitals Rainbow Babies and Children’s Hospital, Department of Pediatric Cardiology, Cleveland, OH; Patricia Elizabeth Thomas, MD, Ochsner Health System, Pediatric Cardiology/Electrophysiology, New Orleans, LA; and Ilana J. Zeltser, MD, The University of Texas at Southwestern Medical Center, Department of Pediatrics / Cardiology, Dallas, TX. In addition, we are grateful to the other referring physicians whose LQTS patients still remain genetically elusive and are under intense investigation to expose their root cause.

**Funding Sources:** This work was supported by the Mayo Clinic Windland Smith Rice Comprehensive Sudden Cardiac Death Program, the Sheikh Zayed Saif Mohammed Al Nahyan Fund in Pediatric Cardiology Research, the Dr. Scholl Fund, and the Hannah M. Wernke Memorial Fund. This project was also supported in part by funding from Mayo Clinic’s Center for Individualized Medicine (CIM).

**Conflict of Interest Disclosures:** MJA is a consultant for Transgenomic. Intellectual property derived from MJA’s research program resulted in license agreements in 2004 between Mayo Clinic Ventures (formerly Mayo Medical Ventures) and PGxHealth (formerly Genaissance Pharmaceuticals and now Transgenomic). However, Transgenomic did not contribute directly to this study in any manner. HMA, DJT, MLW, SM, JME, BWE and MJA have no conflicts of interest.

**References:**


Table 1. Disease-Network Analysis Candidate Gene Ranking.

<table>
<thead>
<tr>
<th>Gene</th>
<th>RANK</th>
<th>ToppGene</th>
<th>Endeavor</th>
<th>Combined</th>
<th>Total Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRDN</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>SLC7A9</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>MEF2A</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>SIRPA</td>
<td>7</td>
<td>5</td>
<td>12</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>PRSS1</td>
<td>4</td>
<td>11</td>
<td>15</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>AQP7</td>
<td>9</td>
<td>7</td>
<td>16</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>CEACAM1</td>
<td>11</td>
<td>6</td>
<td>17</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>FES</td>
<td>6</td>
<td>13</td>
<td>19</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>ENAH</td>
<td>12</td>
<td>9</td>
<td>21</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>MAFA</td>
<td>10</td>
<td>12</td>
<td>22</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>HLA-DQA2</td>
<td>15</td>
<td>8</td>
<td>23</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>ANKRD30A</td>
<td>5</td>
<td>21</td>
<td>26</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>SPEN</td>
<td>8</td>
<td>18</td>
<td>26</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>MADCAM1</td>
<td>27</td>
<td>4</td>
<td>31</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>TUBA3C</td>
<td>19</td>
<td>15</td>
<td>34</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>CHD3</td>
<td>25</td>
<td>10</td>
<td>35</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>NUP188</td>
<td>20</td>
<td>16</td>
<td>36</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>FBXO2</td>
<td>14</td>
<td>23</td>
<td>37</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>MYNN</td>
<td>21</td>
<td>17</td>
<td>38</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>TMPRSS9</td>
<td>16</td>
<td>25</td>
<td>41</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>FLG2</td>
<td>13</td>
<td>29</td>
<td>42</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>PTPN13</td>
<td>29</td>
<td>14</td>
<td>43</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>DNAH1</td>
<td>23</td>
<td>22</td>
<td>45</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>MAMDC4</td>
<td>26</td>
<td>19</td>
<td>45</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>OR4C45</td>
<td>18</td>
<td>28</td>
<td>46</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>OR10H1</td>
<td>17</td>
<td>30</td>
<td>47</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>PRAMEF4</td>
<td>22</td>
<td>26</td>
<td>48</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>PLCXD1</td>
<td>28</td>
<td>20</td>
<td>48</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>NDUFAF7</td>
<td>24</td>
<td>27</td>
<td>51</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td>LYSMD4</td>
<td>30</td>
<td>24</td>
<td>54</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>KRTAP4-8</td>
<td>31</td>
<td>32</td>
<td>63</td>
<td>31</td>
<td>31</td>
</tr>
<tr>
<td>PRB4</td>
<td>32</td>
<td>31</td>
<td>63</td>
<td>32</td>
<td>32</td>
</tr>
</tbody>
</table>
Table 2. Demographics of the Phenotype-Positive/Genotype-Negative LQTS Cohort.

<table>
<thead>
<tr>
<th></th>
<th>LQTS (n=33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)</td>
<td>12/21</td>
</tr>
<tr>
<td>Average Age at Diagnosis (years) + Standard Deviation</td>
<td>23.6 ± 18.7</td>
</tr>
<tr>
<td>Age Range (years)</td>
<td>1 to 65</td>
</tr>
<tr>
<td>Number of Patients Diagnosed at ≤ 10 Years of Age</td>
<td>9</td>
</tr>
<tr>
<td>Number of Patients Diagnosed at &gt; 10 Years of Age</td>
<td>24</td>
</tr>
<tr>
<td>Average QTc (ms) + Standard Deviation</td>
<td>515 ± 56</td>
</tr>
<tr>
<td>Percent (%) with Syncope</td>
<td>42%</td>
</tr>
<tr>
<td>Percent (%) with Cardiac Arrest</td>
<td>33%</td>
</tr>
<tr>
<td>Percent (%) with a Positive Family History</td>
<td>36%</td>
</tr>
</tbody>
</table>
Table 3. Triadin (TRDN) Mutation Positive Patient Summary.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Ethnicity</th>
<th>LQTS Dx Score</th>
<th>QTc (ms)</th>
<th>Symptoms</th>
<th>Age at First CA</th>
<th>Skeletal Myopathy</th>
<th>Family History</th>
<th>Nucleotide Change</th>
<th>Protein Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td>F</td>
<td>African American</td>
<td>7</td>
<td>500</td>
<td>Exertion – induced Syncope/CA</td>
<td>3 years</td>
<td>None</td>
<td>No</td>
<td>Homo. c.del 53_56 ACAG</td>
<td>p.D18fs*13</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>Indian</td>
<td>7.5</td>
<td>490</td>
<td>Exertion – induced Syncope/CA TdP</td>
<td>3 years</td>
<td>Slight proximal muscle weakness</td>
<td>Sibling – CA at age 2</td>
<td>Homo. c.del 572_576 TAAGA</td>
<td>p.K147fs*0</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>Arabic</td>
<td>5.5</td>
<td>480</td>
<td>Exertion – induced Syncope/CA</td>
<td>2 years</td>
<td>None</td>
<td>Cousin – SCD at age 6 years</td>
<td>Homo. c.del 572_576 TAAGA</td>
<td>p.K147fs*0</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>Indian</td>
<td>7</td>
<td>500</td>
<td>Startle / Exertion – induced syncope/CA</td>
<td>23 months</td>
<td>None</td>
<td>No</td>
<td>Homo. c.del 572_576 TAAGA</td>
<td>p.K147fs*0</td>
</tr>
</tbody>
</table>

*Patient for the WES pedigree. F=female, M=male, Dx=diagnosis, CA=cardiac arrest, TdP=torsades de pointes, SCD=sudden cardiac death, Homo=homozygous, Het=heterozygous
Table 4. Summary of Triadin Knockout (TKO) Syndrome

<table>
<thead>
<tr>
<th>Triadin Knockout Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inheritance Pattern:</strong></td>
</tr>
<tr>
<td>- Autosomal Recessive (homozygous or compound heterozygous TRDN mutations)</td>
</tr>
<tr>
<td><strong>Electrocardiographically:</strong></td>
</tr>
<tr>
<td>- Extensive T-wave inversions in precordial leads V1-V4</td>
</tr>
<tr>
<td>- Consistent or transient QT prolongation</td>
</tr>
<tr>
<td><strong>Phenotypic Expression:</strong></td>
</tr>
<tr>
<td>- Severe disease expression of exercise-induced syncope/cardiac arrest in early childhood (≤ 5 years), often with first presentation during the second year of life</td>
</tr>
<tr>
<td>- Possible non-cardiac involvement with mild to moderate skeletal muscle weakness</td>
</tr>
<tr>
<td><strong>May Require Aggressive Therapy:</strong></td>
</tr>
<tr>
<td>- Beta blocker*</td>
</tr>
<tr>
<td>- Implantation of an internal cardioverter defibrillator (ICD)</td>
</tr>
<tr>
<td>- Left cardiac sympathetic denervation (LCSD) surgery</td>
</tr>
</tbody>
</table>

* A calcium channel blocker like nifedipine or verapamil may be a therapeutic consideration

Figure Legends:

**Figure 1.** WES and Familial Genomic Triangulation for the Elucidation of a Novel Genetic Substrate for LQTS. Depicted is the A) LQTS pedigree with a presumed sporadic/autosomal recessive inheritance pattern showing the case-parent trio (yellow triangle) and the affected index case (black circle) and 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, and D) Sanger sequencing confirmation of the TRDN mutation. The yellow triangle with white square (unaffected father), white circle (unaffected mother), and black circle (affected index case) represent the case-parent trio that 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.

**Figure 2.** Pedigree and Mutation Summary for Patients 2-5. Shown in the top box are the pedigrees for index cases 2-4 who were all identified with the same exact homozygous K147fs*0 frame-shift mutation. Black circles represent affected individuals. White circles/squares represent unaffected individuals. The (+) symbol represents a mutated allele and the (–) symbol represents a normal allele. NA = not available. Also shown are the Sanger sequencing chromatograms (from a normal control and representative patient) that reveal the TRDN mutation. The mutation is localized on a linear topology figure of triadin. The bottom box illustrates the pedigree for patient 5 who was identified as compound heterozygous for TRDN frame-shift mutations (N9fs*5 and K147fs*0).

**Figure 3.** Representative Electrocardiograms for Patients 2-5. Electrocardiograms displaying the common electrocardiographic signature of extensive T-wave inversion in precordial leads V1-V4 with QT-prolongation.

**Figure 4.** The Effect of Age on the Percent Containing a TRDN Mutation. Depicted is a graph comparing the percent containing a TRDN mutation identified in patients \( \leq 10 \) years of age versus patients older than 10 years.
Figure 1

(a) Pedigree of family with a case of atrial fibrillation. The black circle represents the index case, and the black square represents the unaffected parent.

(b) Electrocardiogram showing atrial fibrillation.

(c) Sporadic Model vs. Recessive Model for atrial fibrillation.

- **Sporadic Model**
  - Variants identified by WES: 123,201 variants
  - Filter 1: Include variants with a call quality score ≥ 20 and present in genes outside the top 1% of genes with high variability (116,276 variants)
  - Filter 2: Include only ultra-rare variants (allele frequency ≤ 0.01% in publicly available databases) (8,721 variants)
  - Filter 3: Include only non-synonymous variants (942 variants)
  - Filter 4: Include only variants absent in both parents (26 variants - 19 genes)

- **Recessive Model**
  - Variants identified by WES: 123,201 variants
  - Filter 1: Include variants with a call quality score ≥ 20 and present in genes outside the top 1% of genes with high variability (116,276 variants)
  - Filter 2: Include only rare variants (allele frequency ≤ 1% in publicly available databases) (15,726 variants)
  - Filter 3: Include only non-synonymous variants (2,273 variants)
  - Filter 4: Filter for variants present as homozygotes (5 variants - 5 genes) or compound heterozygotes (18 variants - 9 genes)

(d) Genotype analysis for affected individuals:

- Unaffected Father: ATAGACAAGCAAAAATGG
  - ATAGACAAGCAAAAATGG

- Normal Control: ATAGCAAAAAATGGATCT
  - ATAGCAAAAAATGGATCT

- Index Case - Homozygous c.del53_56 ACAG
  - ATAGCAAAAAATGGATCT
  - ATAGCAAAAAATGGATCT

- Unaffected Mother: ATAGACAAGCAAAAATGG
  - ATAGCAAAAAATGGATCT

The figure explains the genetic basis of atrial fibrillation, highlighting the differences between sporadic and recessive models and the specific variants identified.
Figure 4

- For ≤ 10 years of age (n=10), the yield is 50%.
- For > 10 years of age (n=24), the yield is 0%.

P < 0.001
Homozygous/Compound Heterozygous Triadin Mutations Associated with Autosomal Recessive Long QT Syndrome and Pediatric Sudden Cardiac Arrest: Elucidation of Triadin Knockout Syndrome

Helene M. Altmann, David J. Tester, Melissa L. Will, Sumit Middha, Jared M. Evans, Bruce W. Eckloff and Michael J. Ackerman

Circulation. published online April 28, 2015;
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2015 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/early/2015/04/28/CIRCULATIONAHA.115.015397

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/