Circulation: Genomic and Precision Medicine

ORIGINAL ARTICLE

International Triadin Knockout Syndrome Registry

The Clinical Phenotype and Treatment Outcomes of Patients With Triadin Knockout Syndrome

BACKGROUND: Triadin knockout syndrome (TKOS) is a rare, inherited arrhythmia syndrome caused by recessive null mutations in *TRDN*-encoded cardiac triadin. Based previously on 5 triadin null patients, TKOS has been characterized by extensive T-wave inversions, transient QT prolongation, and severe disease expression of exercise-induced cardiac arrest in early childhood refractory to conventional therapy.

METHODS: We have established the International Triadin Knockout Syndrome Registry to include patients who have genetically proven homozygous/compound heterozygous *TRDN* null mutations. Clinical/genetic data were collected using an online survey generated through REDCap.

RESULTS: Currently, the International Triadin Knockout Syndrome Registry includes 21 patients (11 males, average age of 18 years) from 16 families. Twenty patients (95%) presented with either cardiac arrest (15, 71%) or syncope (5, 24%) at an average age of 3 years. Mild skeletal myopathy/proximal muscle weakness was noted in 6 (29%) patients. Of the 19 surviving patients, 16 (84%) exhibit T-wave inversions, and 10 (53%) have transient QT prolongation > 480 ms. Eight of 9 patients had ventricular ectopy on exercise stress testing. Thirteen (68%) patients have received implantable defibrillators. Despite various treatment strategies, 14 (74%) patients have had recurrent breakthrough cardiac events.

CONCLUSION: TKOS is a potentially lethal disease characterized by T-wave inversions in the precordial leads, transient QT prolongation in some, and recurrent ventricular arrhythmias at a young age despite aggressive treatment. Patients displaying this phenotype should undergo *TRDN* genetic testing as TKOS may be a cause for otherwise unexplained cardiac arrest in young children. As gene therapy advances, enrollment into the International Triadin Knockout Syndrome Registry is encouraged to better understand TKOS and to ready a well-characterized cohort for future *TRDN* gene therapy trials.

Daniel J. Clemens, BS
David J. Tester, BS
John R. Giudicessi, MD, PhD
J. Martijn Bos, MD, PhD
Ram K. Rohatgi, MD
Dominic J. Abrams, MD, MRCP, MBA
Seshadri Balaji, MBBS, MRCP, PhD
Lia Crotti, MD, PhD
Julien Faure, PhD
Carlo Napolitano, MD, PhD
Silvia G. Priori, MD, PhD
Vincent Probst, MD
Caroline Rooryck-Thambo, MD, PhD
Nathalie Roux-Buisson, MD, PhD
Frederic Sacher, MD
Peter J. Schwartz, MD
Michael J. Silka, MD
Mark A. Walsh, MD
Michael J. Ackerman, MD, PhD

Key Words: genetics ■ human ■ long QT syndrome ■ pediatrics ■ phenotype

https://www.ahajournals.org/journal/circgen

Sudden cardiac death produces a major worldwide public health burden, with an estimated annual occurrence ranging from 180 000 to 450 000 in the United States[1] and as many as 3.7 million deaths worldwide.[2] Sudden death in the young has a devastating and profound societal impact with ≈2000 to 5000 young people between 1 and 35 years of age experiencing sudden cardiac death yearly in the United States alone.[3] Potentially lethal cardiac arrhythmia syndromes, such as long QT syndrome (LQTS), catecholaminergic polymorphic ventricular tachycardia (CPVT), and idiopathic ventricular fibrillation underlie a significant portion of sudden death in the young.

Recently, a few small cohort studies and case reports have identified *TRDN*-encoded cardiac triadin as an underlying cause for some patients diagnosed originally as LQTS, CPVT, or idiopathic ventricular fibrillation.[4–8] In 2012, triadin null mutations were first published as an underlying cause for recessively inherited arrhythmogenic disease when 2 out of 97 (2%) patients diagnosed with CPVT, yet genotype negative for *RYR2* and *CASQ2*, were discovered to have either homozygous or compound heterozygous triadin null mutations.[4] Three subsequent case reports have also identified triadin null mutations in patients originally diagnosed with CPVT or idiopathic ventricular fibrillation.[5–7]

In 2015, Altmann et al[8] discovered either homozygous or compound heterozygous frameshift mutations in *TRDN* to be the underlying cause of disease in 5 patients diagnosed originally with genetically elusive LQTS. These subjects had a common electrocardiographic and clinical phenotype with atypical feature of both LQTS and CPVT. Consequently, triadin knockout syndrome (TKOS) was coined rather than *TRDN*-mediated LQTS/LQT17 or *TRDN*-mediated CPVT/CPVT5.[8] Remarkably, all 5 patients displayed either consistent or transient QT prolongation, exhibited extensive T-wave inversions (TWI) in precordial leads V_1 through V_4, and experienced severe disease expression of exercise-induced sudden cardiac arrest (SCA) in early childhood, with most experiencing recurrent breakthrough cardiac events (BCEs), despite aggressive therapy. Additionally, 2 of the 5 patients displayed mild skeletal muscle weakness.[8]

Given the potentially lethal nature of this syndrome, we set out to better define the phenotype and treatment outcomes of patients with TKOS through the establishment of the International Triadin Knockout Syndrome Registry (ITKOSR).

METHODS

To prevent the reidentification of patients included in this study, individual patient data will not be made available to other researchers. This study was approved by the Mayo Clinic Institutional Review Board which included a waiver for informed patient consent. The detailed methods are included in the Data Supplement.

RESULTS

Patient Demographics

Overall, we identified 21 patients from 16 unrelated families that met the criteria for inclusion into the ITKOSR (Tables 1 and 2). Of the 21 patients, 14 have been described previously in the literature.[4–8] Overall, there were 11 (52%) males and 10 (48%) females. The average age of enrollment into the registry was 18±14 years of age (ranging from 3 to 45 years). Patients came from a variety of ethnic backgrounds including white (8/21, 38%), Indian, (4, 19%), Latino (3, 14%), African (2, 9%), and Arabic (2, 9%), along with 2 (9%) patients who were half white and half African (Table 2). Before their TKOS diagnosis, patients were diagnosed with either LQTS (8/21, 38%), CPVT (7, 33%), idiopathic ventricular fibrillation (4, 19%), exercise-induced syncope (1, 5%), or unexplained sudden cardiac death (1, 5%; Figure I in the Data Supplement).

Genetics

All patients enrolled in the ITKOSR have either homozygous (12/21, 57%) or compound heterozygous (9/21, 43%) triadin null variants (Table 3). We identified 13 unique pathogenic variants or likely pathogenic variants in *TRDN* (Figure 1; Table I in the Data Supplement). Of these unique variants, 7 were discovered in previously published cases of TKOS,[4–8] whereas 6 are being reported here for the first time. The vast majority of these variants were either frameshift (6, 46%), nonsense (2, 15%), or splice site-altering (2, 15%) variants that lead to an early stop codon and nonsense-mediated decay. Four of the pathogenic variants (p.D18fs*13 [2 families], p.N9fs*5 [2 families], p.K147fs*0 [5 families], p.Q205* [2 families]) have been observed in multiple unrelated families. Additionally, 2 (15%) pathogenic missense (p.T59R and p.T59M) variants and 1 (8%) large copy number variant deletion of exon 2 were also discovered (Figure 1; Table I in the Data Supplement). The transmembrane domain residing p.T59R missense variant has been characterized previously and shown to result in an unstable protein that undergoes proteasomal degradation.[4] Presumably, the p.T59M missense variant occurring at the same amino acid residue as p.T59R also produces an unstable protein that undergoes proteasomal degradation and ultimately results in a null allele. The homozygous deletion of exon 2 which encodes for most of the N terminus and all of the transmembrane domain of triadin (Figure 1) was identified in a single patient and recently reported by O'Callaghan et al.[7]

Table 1. Clinical Characteristics of Patients With TKOS

Demographics	
Patients	21
Unique families	16
Females	10 (48)
Age at enrollment, y	18±14
Symptoms	
Any cardiac event	20 (95)
Sudden cardiac arrest	17 (81)
Sudden cardiac death	2 (9)
Age at first event, y	3±2
Skeletal myopathy/proximal muscle weakness	6 (29)
Clinical features (n=19)	
Mean QTc, ms	472±34
T-wave inversions	16 (84)
V_1 through V_3	6 (32)
V_1 through V_4	4 (21)
V_1 through V_5	1 (5)
Other	5 (26)
Normal echocardiogram (n=16)	16 (100)
Ectopy upon stress testing (n=9)	8 (89)
Treatments (n=19)	
β- blockers (nadolol, propranolol, bisoprolol)	19 (100)
Sodium channel blockers (flecainide, mexiletine)	6 (32)
Calcium channel blocker (verapamil)	1 (5)
LCSD	9 (47)
RCSD	3 (16)
Device implantation	14 (74)
ICD	13 (68)
Pacemaker	1 (5)
Outcomes (n=19)	
Experienced BCE	14 (74)
1 BCE	1 (5)
2–5 BCEs	8 (42)
6–10 BCEs	2 (11)
>10 BCEs	3 (16)

Values are n (%) or mean±SD. BCE indicates breakthrough cardiac event; ICD, implantable cardioverter defibrillator; LCSD, left cardiac sympathetic denervation; QTc, corrected QT interval; RCSD, right cardiac sympathetic denervation; and TKOS, triadin knockout syndrome.

Symptoms

Overall, 20 out of 21 (95%) patients were symptomatic, having had at least 1 cardiac event. Patients who had a recorded date of first cardiac event (18, 86%) presented at a young age, with their first event occurring at an average age of 3±2 years (Table 1). Additionally, 83% of symptomatic patients presented by age 5 years, and all by 10 years of age. Syncope was the sentinel event in 5 (24%) patients, whereas 15 (71%) patients presented initially with SCA. However, 2 of the 5 patients who presented initially with syncope went on to have an SCA. Thus, 17 out of 21 (81%) patients have had an SCA (Tables 1 and 2).

The most common trigger was physical exertion which was associated with at least one cardiac event in 17 of the 21 (81%) patients. Other triggers included auditory stimuli, fear, and sleep. However, a large number of events were not associated with a specific trigger. In addition to their severe cardiac symptoms, 6 (29%) patients also presented with mild skeletal myopathy or slight proximal muscle weakness (Tables 1 and 2). Currently, 19 patients are still living, whereas 2 (patients no. 12 and no. 17, Table 2) have died after cardiac events.

Clinical Evaluation

All 19 surviving TKOS patients have had at least one 12-lead ECG. Of these, 10 patients (53%) exhibited a heart rate corrected QT interval (QTc) >480 ms on at least 1 ECG. Although the QT prolongation has been transient in 9 of the 10 patients, 1 patient does manifest a consistently prolonged QTc (patient no. 21, Table 2). Furthermore, 16 out of 19 (84%) surviving patients exhibit TWIs, of which 13 (68%) have TWIs in the precordial leads with the majority showing them in leads V_1 through V_3 or V_1 through V_4 (Tables 1 and 2) as shown in Figure 2A and Figure II in the Data Supplement.

All 16 patients tested had a normal echocardiogram suggesting a structurally normal heart (Tables 1 and 2). Of the 9 patients that had an exercise stress test, 8 (89%) exhibited ventricular ectopy at an average onset heart rate of 108±24 bpm (Table 1). The extent of exercise-induced ectopy seen in these patients was premature ventricular contractions in isolation, bigeminy, couplets, and triplets (Figure 2B; Table 2). None of the patients had either nonsustained ventricular tachycardia or bidirectional ventricular tachycardia.

Treatment

All 19 surviving patients are being treated with β-blocker therapy. The vast majority are currently on either nadolol (14, 74%) or propranolol (4, 21%), whereas 1 patient (5%) is being treated with Bisoprolol and 1 patient (5%) with metoprolol. In addition to β-blocker therapy, 6 (32%) patients have had a sodium (flecainide, n=5; mexiletine, n=1) or calcium (verapamil, n=1) channel blocker added to their treatment regimen. Left cardiac sympathetic denervation (LCSD) was completed in 9 (47%) patients, and 3 (15%) have also undergone a subsequent right cardiac sympathetic denervation (Table 1).

A total of 14 (74%) patients have undergone device implantation. Thirteen (68%) have had an implantable cardioverter defibrillator implanted, and 1 patient (5%)

Table 2. Patient-Specific Clinical Characteristics

Patient	Family	Sex	Ethnicity	Age at Enrollment, y	Symptomatic	Age at First Event, y	Sentinel Event	Sudden Cardiac Arrest	Skeletal Myopathy	QTc, ms	T-Wave Inversions	Stress Test	Echocardiogram
1[5]	1	Female	White	9	Yes	4	SCA	Yes	No	440	V_1 through V_3	PVCs in couplets	Normal
2[5]	1	Female	White	10	Yes	6	Syncope	No	No	442	V_1 through V_3	PVCs in isolation, bigeminy	…
3[5]	1	Female	White	7	No	…	…	…	No	405	V_1 through V_3	…	…
4[8]	2	Male	White	7	Yes	1	SCA	Yes	Yes	475	V_1 through V_3	…	Normal
5[8]	3	Female	African	14	Yes	1	Syncope	Yes	No	496	V_1 through V_4	PVCs in isolation, bigeminy, couplets	Normal
6[8]	4	Female	Indian	11	Yes	2	SCA	Yes	Yes	500	V_1 through V_3	…	Normal
7[8]	5	Male	Arabic	13	Yes	2	SCA	Yes	No	480	V_1 through V_4	…	Normal
8[8]	6	Female	Indian	9	Yes	1	SCA	Yes	No	504	V_1 through V_4	PVCs in isolation, bigeminy	Normal
9	7	Male	White	41	Yes	2	SCA	Yes	No	442	V_1	PVCs in bigeminy, couplets	Normal
10[6]	8	Male	African/ White	16	Yes	1	SCA	Yes	Yes	490	V_1 through V_3	…	Normal
11[6]	8	Female	African/ White	6	Yes	1	SCA	Yes	Yes	500	V_1 through V_4	…	Normal
12	9	Female	Indian	…	Yes	3	SCA	Yes	No	…	…	…	…
13	10	Male	White	21	Yes	2	SCA	Yes	No	470	None	No ectopy	…
14	11	Male	Indian	41	Yes	10	Syncope	Yes	No	445	II	PVCs in isolation, bigeminy, couplets	Normal
15[4]	12	Male	White	45	Yes	N/A	Syncope	No	No	427	I+aVR+aVL	PVCs in triplets	Normal
16[4]	12	Male	White	45	Yes	N/A	Syncope	No	Yes	432	None	PVCs in triplets	…
17[4]	13	Male	African	…	Yes	2	SCA	Yes	No	…	…	…	Normal
18	14	Female	Hispanic	13	Yes	3	SCA	Yes	No	488	III	…	Normal
19	14	Male	Hispanic	8	Yes	5	SCA	Yes	No	539	None	…	Normal
20	15	Female	Hispanic	11	Yes	2	SCA	Yes	No	500	III+aVF+V_3 through V_5	…	Normal
21[7]	16	Male	Arabic	3	Yes	1	SCA	Yes	Yes	490	V_1 through V_5	…	Normal

Reference numbers indicate where that TKOS patient was first published. PVC indicates premature ventricular contraction; QTc, corrected QT interval; SCA, sudden cardiac arrest; and TKOS, triadin knockout syndrome.

underwent placement of a permanent pacemaker to allow for increased β-blocker dose (Table 1).

Outcomes

Despite these various treatment strategies, 14 out of 19 (74%) surviving TKOS patients have had on-therapy BCEs. In fact, all but one of these patients (patient no. 19) has had >1 BCE, and 5 (26%) have had >5 BCEs (Table 1).

The majority of TKOS patients have not only had multiple BCEs but also have experienced those events while on different combinations of therapy. Most surviving patients (17, 89%) were initially treated with either β-blocker ther-

Table 3. Patient-Specific Pathogenic/Likely Pathogenic Variants

Patient	Family	Homozygous/ Compound Heterozygous	Nucleotide Change 1	Amino Acid Change 1	Variant Type	Nucleotide Change 2	Amino Acid Change 2	Variant Type
1[5]	1	Compound heterozygous	c.613C>T	p.Q205*	Nonsense	c.22+29A>G	p.N9fs*5	Intronic/splice-site
2[5]	1	Compound heterozygous	c.613C>T	p.Q205*	Nonsense	c.22+29A>G	p.N9fs*5	Intronic/splice-site
3[5]	1	Compound heterozygous	c.613C>T	p.Q205*	Nonsense	c.22+29A>G	p.N9fs*5	Intronic/splice-site
4[8]	2	Compound heterozygous	c.438_442delTAAGA	p.K147fs*0	Frameshift	c.22+29A>G	p.N9fs*5	Intronic/splice-site
5[8]	3	Homozygous	c.53_56delACAG	p.D18fs*13	Frameshift	…	…	…
6[8]	4	Homozygous	c.438_442delTAAGA	p.K147fs*0	Frameshift	…	…	…
7[8]	5	Homozygous	c.438_442delTAAGA	p.K147fs*0	Frameshift	…	…	…
8[8]	6	Homozygous	c.438_442delTAAGA	p.K147fs*0	Frameshift	…	…	…
9	7	Homozygous	c.423delA	p.E142fs*33	Frameshift	…	…	…
10[6]	8	Compound heterozygous	c.del53_56ACAG	p.D18fs*13	Frameshift	c.502G>T	p.E168*	Nonsense
11[6]	8	Compound heterozygous	c.del53_56ACAG	p.D18fs*13	Frameshift	c.503G>T	p.E168*	Nonsense
12	9	Homozygous	c.438_442delAAGA	p.K147fs*0	Frameshift	…	…	…
13	10	Homozygous	c.545_546insA	p.K182fs*10	Frameshift	…	…	…
14	11	Homozygous	c.420delA	p.K140fs*34	Frameshift	…	…	…
15[4]	12	Compound heterozygous	c.176C>G	p.T59R	Missense	c.613C>T	p.Q205*	Nonsense
16[4]	12	Compound heterozygous	c.176C>G	p.T59R	Missense	c.613C>T	p.Q205*	Nonsense
17[4]	13	Homozygous	c.53_56delACAG	p.D18fs*13	Frameshift	…	…	…
18	14	Homozygous	c.618delG	p.A208fs*15	Frameshift	…	…	…
19	14	Homozygous	c.618delG	p.A208fs*15	Frameshift	…	…	…
20	15	Compound heterozygous	c.232+2T>A	…	Intronic/splice-site	c.176C>T	p.T59M	Missense
21[7]	16	Homozygous	Exon 2 Deletion	…	Large deletion	…	…	…

Reference numbers indicate where that TKOS patient was first published. TKOS indicates triadin knockout syndrome.

apy alone or in combination with a sodium or calcium channel blocker (drug therapy). While on drug therapy alone, 12/17 (71%) patients had at least 1 on-prescription therapy BCE (Figure 3A). These patients had anywhere from 1 to >10 BCEs. Among these 17 patients, the exact duration of follow-up was available for 14. Over a total of 76 years of follow-up, these patients experienced 53 BCEs for a yearly event rate of 0.70 BCEs/y (Figure 3B). Compliance issues may have played a role in a small number of these events. However, the vast majority of patients have been compliant with their pharmacological therapy.

After having a BCE, 6 patients went on to have an LCSD. Additionally, 2 patients (patients no. 6 and no. 14) were initially treated with drug therapy and LCSD. Despite the use of drug therapy and LCSD, these 8 (100%) patients all experienced BCEs (ranging from 1 to 3 events each; Figure 3A). Among these 8 patients, follow-up data were available for 7 patients where 14 BCEs have occurred over 59 follow-up years, yielding a yearly event rate of 0.24 BCEs/y (Figure 3B). Two patients treated with drug therapy and LCSD also underwent right cardiac sympathetic denervation (patients no. 5 and no. 18), and 1 patient went straight from drug therapy to bilateral denervation (patient no. 19, Figure 3A). To this point, 1 of the 3 (33%) patients treated with drug therapy plus LCSD and right cardiac sympathetic denervation has experienced BCEs (Figure 3A).

Based on the 12-lead ECG yielding their longest recorded QTc, it is noteworthy that patients with a BCE on average have a significantly longer QTc (487±25 ms, n=14) than those that have not had any BCEs (429±15 ms, n=5; $P<0.0001$; Figure 4).

DISCUSSION

Since 2012, when mutations in *TRDN* were first discovered to be an underlying cause of disease,[4] only a small

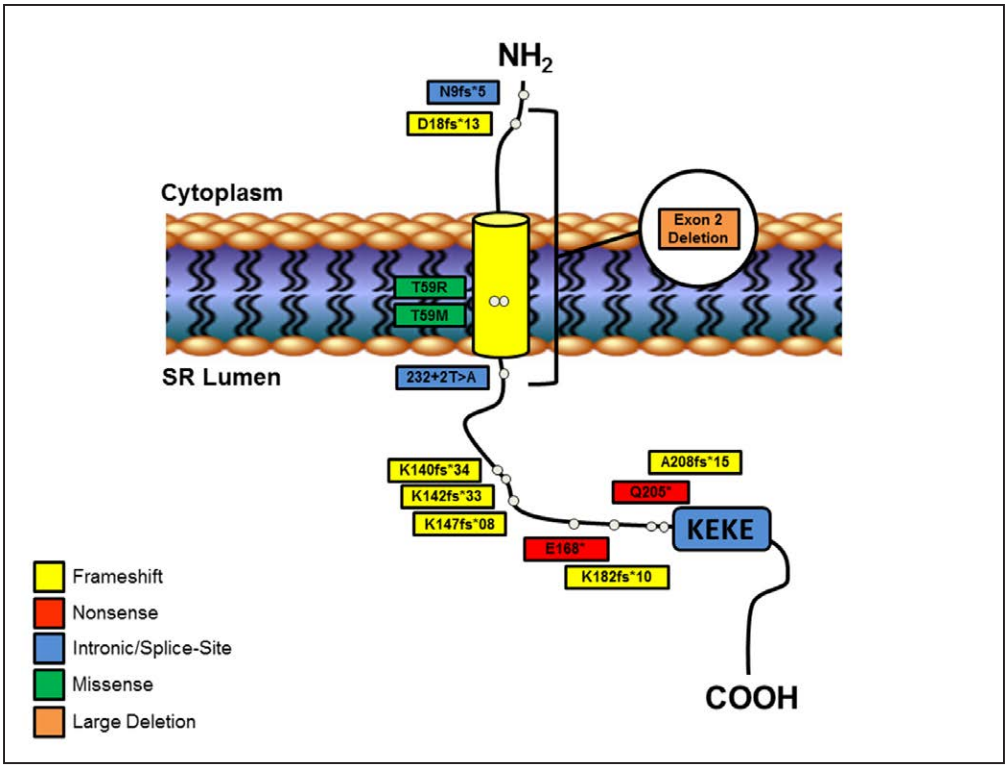

Figure 1. Cardiac triadin topology with location of triadin knockout syndrome (TKOS)-causative variants.
Depicted is a schematic representation of *TRDN*-encoded cardiac triadin with all 13 of the known TKOS-causative variants. Each white dot represents the location of a corresponding pathogenic variant. The large bracket depicts the region of the protein that is missing because of the deletion of exon 2. The KEKE domain is the region known to be responsible for the interaction and binding of both calsequestrin and RyR2 (amino acids 210–224). SR indicates sarcoplasmic reticulum.

number of reports have been put forward describing what we now refer to as TKOS.[5–8] Similar to what has previously been established for LQTS and other arrhythmia syndromes,[9] we set out to collect comprehensive clinical data from a larger number of TKOS patients through an international registry to better understand the natural history, clinical features, and treatment outcomes of patients with this potentially lethal disease. To do this, we have established the ITKOSR and now present the first comprehensive study of the clinical phenotype and treatment outcomes of the first 21 patients enrolled in the ITKOSR.

Triadin is a critical protein within the cardiac calcium release unit complex where the L-type calcium channel (LTCC) is juxtaposed to the RyR2 (type 2 ryanodine receptor) calcium release channel on the junctional sarcoplasmic reticulum.[10,11] This complex is responsible for mediating calcium sensing and proper excitation-contraction coupling in the heart. Triadin is known to bind multiple proteins within the calcium release unit including RyR2, Casq2 (calsequestrin 2) and Jph2 (junctophilin 2) and helps mediate proper calcium release from the sarcoplasmic reticulum through RyR2, as well as structural stabilization of the calcium release unit.[11,12]

The loss of triadin because of either homozygous or compound heterozygous null mutations leads to a particularly malignant and potentially lethal phenotype. All but one of the patients in the registry (95%) has had at least 1 cardiac event. Although this rate is in itself extremely high, what is particularly concerning is that 81% of TKOS patients have experienced SCA. This rate is comparable to even the most malignant arrhythmia syndromes, such as Jervell and Lange-Nielsen syndrome,[13] Timothy syndrome,[14] and the calmodulinopathies (Table 4).[18] Similar to these other malignant arrhythmia syndromes, TKOS patients present at a young age, usually before 5 years of age, which is much younger than most patients with LQTS or CPVT (Table 4). However, it should be noted that we cannot yet exclude the possibility that the severity of these cases is due in part to the fact that these are the initial cases of TKOS to be described and that, with time, milder cases may be diagnosed as has been the case with most other arrhythmia disorders.[23]

Additionally, similar to other distinct arrhythmia syndromes that display noncardiac symptoms, such as JLNS,[13] TS,[14] and the calmodulinopathies,[18] 29% of TKOS patients exhibit a mild skeletal myopathy or slight proximal muscle weakness (Table 4). Alternative splicing of triadin mRNA results in tissue-specific isoforms of triadin found in both cardiac and skeletal muscle.[24–26] Because these different isoforms are homologous up to amino acid 264,[27] all of the mutations discovered to date resulting in cardiac triadin null status also re-

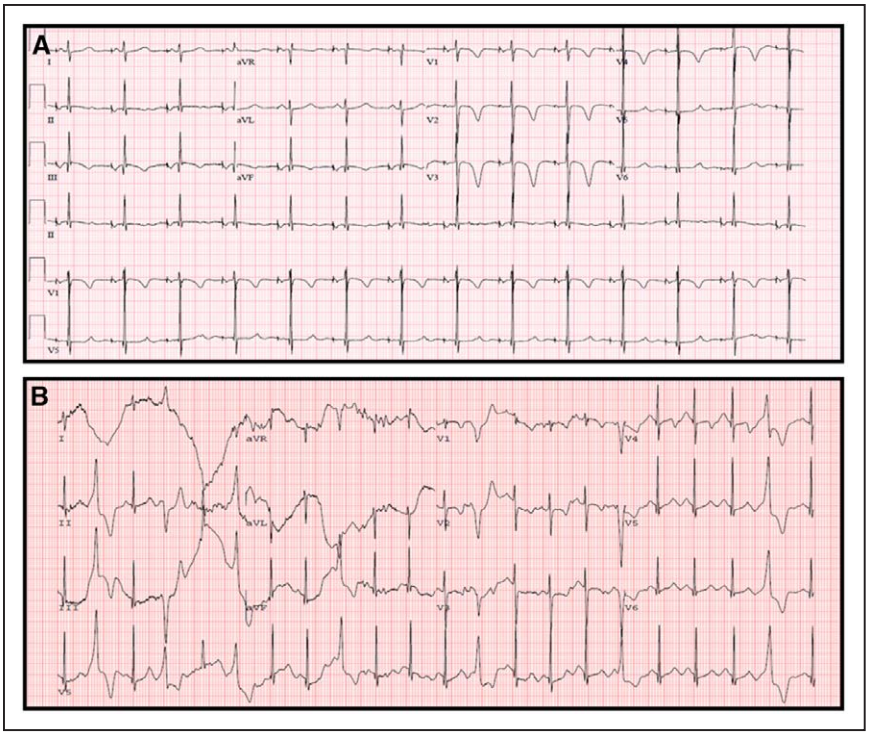

Figure 2. Electrocardiographic hallmark features of triadin knockout syndrome.
A, Representative resting 12-lead ECG displaying T-wave inversions in precordial lead V_1-V_4. **B**, Representative 12-lead ECG from an exercise stress test displaying frequent ventricular ectopic activity.

sult in patients being skeletal muscle triadin null.[28] Why more TKOS patients do not display a skeletal phenotype remains unknown, but it is possible that we are underestimating the number of patients with a skeletal myopathy because of the fact that the phenotype is mild (at least as ascertained by genetic cardiologists) and most of these patients have not been evaluated by a neuromuscular specialist.

TKOS patients also show several interesting features on their ECG. Over 50% of TKOS patients exhibit QTc prolongation of >480 ms. Because this QT prolongation was only transiently observed in 90% of these patients, it

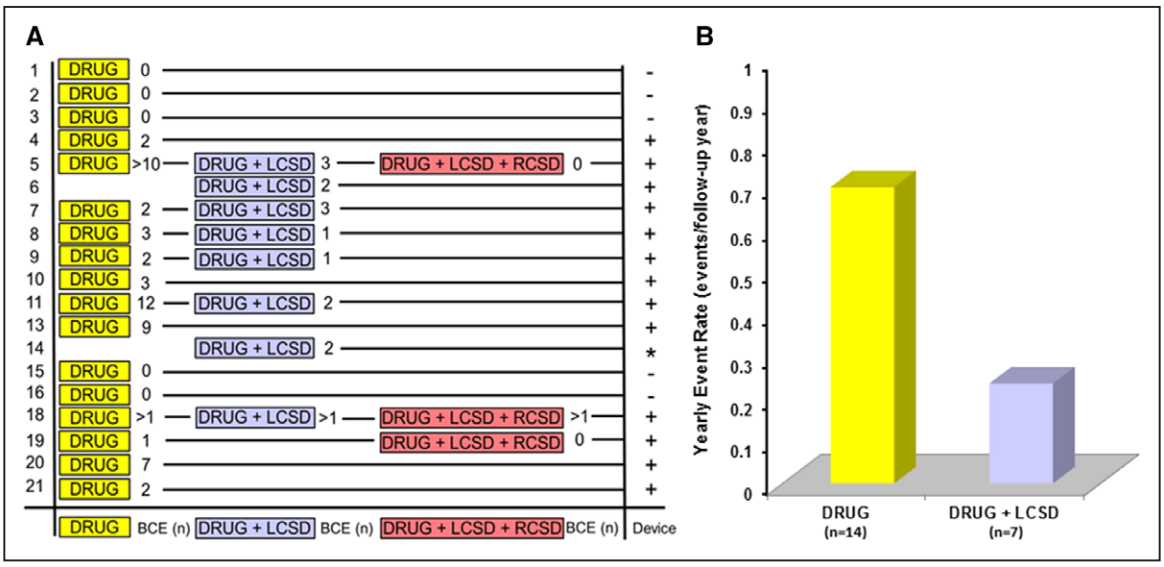

Figure 3. Treatment outcomes in patients with triadin knockout syndrome (TKOS).
A, Depicted are the treatment outcomes of each TKOS patient in the International Triadin Knockout Syndrome Registry (ITKOSR). Each number in the left-hand column represents each unique patient. For example, patient no. 1 in Figure 4 is patient no. 1 in Table 2. The first treatment listed for each patient represents their initial treatment strategy. The following number is the number of breakthrough cardiac events (BCEs) that the patient had while on that specific treatment strategy. This is then the same for each subsequent treatment listed. The last treatment listed for a patient is their current treatment strategy. The symbols in the right-hand column represent whether that patient has been implanted with a device (**+**, implantable cardioverter defibrillator; *, pacemaker; –, no device). For example, patient no. 7 was initiated on drug therapy and had 2 BCEs. Patient no. 7 then underwent left cardiac sympathetic denervation (LCSD) and had 3 more BCEs and is currently being treated with drug therapy and LCSD. This patient also has an implantable cardioverter defibrillator. **B**, The yearly event rate was calculated and expressed as events per follow-up year for patients with exact follow up time available on either drug therapy (0.70 BCEs/y, n=14) or drug therapy and LCSD (0.24 BCEs/y, n=7). DRUG denotes β-blocker or β-blocker+sodium/calcium channel blocker. RCSD indicates right cardiac sympathetic denervation.

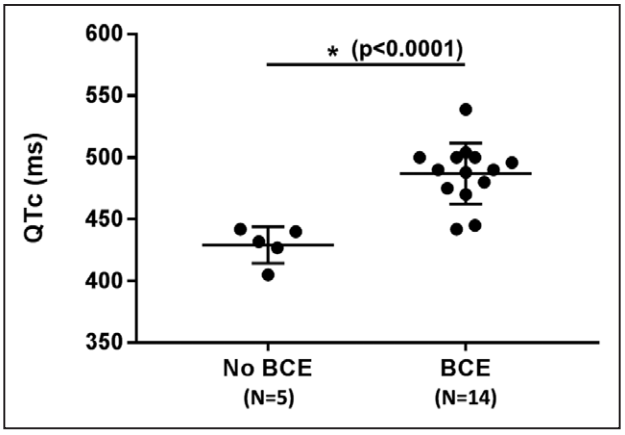

Figure 4. Comparison of corrected QT interval (QTc) in triadin knockout syndrome (TKOS) patients who have or have not experienced breakthrough cardiac events.

Patients who have (breakthrough cardiac event [BCE]) or have not (No BCE) experienced BCEs were compared according to their QTc (ms). Patients who have had BCEs had a significantly longer QTc (487±25 ms; n=14) compared to those who have not (429±15 ms; n=5). Each dot represents an individual patient. Data are presented as mean±SD. **P*<0.0001 by Students *t* test.

may be important for clinicians treating TKOS patients to collect serial ECGs. Interestingly, based on their longest QTc on record, TKOS patients that have experienced BCE had a significantly longer QTc than TKOS patients that have not broken through their therapy. Whether QTc is surrogate marker for those patients at greatest risk for experiencing a BCE remains to be determined. Our cohort as a whole displays an average QTc of 472 ms which is similar to previously published cohorts of patients with LQT1-3[15–17] and is significantly longer than what has been observed in cohorts of patients with CPVT (Table 4).[20,21] Conversely, 89% of TKOS patients exhibit induced ectopy at peak exercise on stress testing which is known to be a hallmark feature of CPVT[19–22] and is rarely, if ever, observed in patients with LQTS (Table 4).

Another common ECG feature among TKOS patients is the presence of extensive TWIs in the precordial leads. Although TWIs in the precordial leads can be common during childhood in healthy subjects,[29] the consistent extension of these inversions into leads V_3, and especially V_4 and V_5, represents an abnormal ECG phenotype. Whether or not TWIs continue to be present into adulthood remains to be seen, but when combined with other clinical features of TKOS, TWIs could serve as a distinct diagnostic criterion that separates TKOS from other arrhythmia syndromes.

Despite the fact that patients with triadin null status have been diagnosed previously with a variety of cardiac arrhythmia disorders, this clinical data provides substantial evidence suggesting that these patients do not have typical LQTS or CPVT, but instead suffer from a distinct overlap disorder that displays clinical features of multiple arrhythmia syndromes, as well as features unique to TKOS.

Multiple studies that have been completed in TKO mice also corroborate these findings.[30,31] In mice, ablation of triadin causes perturbations in the structure of the calcium release unit and a loss of colocalization of the LTCC to RyR2 leading to increased sarcoplasmic reticulum calcium load and impaired LTCC inactivation which in turn causes both ventricular arrhythmias and decreased muscle strength.[30,31] Therefore, it is hypothesized that in TKOS patients, sarcoplasmic reticulum calcium overload could be leading to delayed afterdepolarizations similar to CPVT, and delayed LTCC inactivation could cause a prolonged action potential and early afterdepolarizations, both of which are precursors of ventricular arrhythmias.[12] However, the definitive mechanism by which loss of triadin leads to such a severe patient phenotype has yet to be defined by characterization in a human-specific model.

It has also become clear from our clinical data that the treatment strategies which are currently available are not adequate to effectively manage patients with TKOS. β-blockers, which are highly effective in treating patients with LQTS or CPVT,[32,33] failed to prevent cardiac events in 71% of TKOS patients. Additionally, whereas some patients have remained event free on combination β-blocker and flecainide therapy, which has been shown both clinically and experimentally to improve arrhythmia control in patients with CPVT,[34,35] others who have been treated with either flecainide or mexiletine have gone on to have multiple BCEs. One patient is currently being treated with the calcium channel blocker verapamil along with β-blocker and flecainide. Although it is difficult to draw any conclusions about the therapeutic potential of calcium channel blockers in TKOS from this single patient, these drugs do provide a potential alternative when considering the proposed mechanism of delayed LTCC inactivation.[8,30] However, the effectiveness of drugs, such as verapamil or nifedipine, may be limited because of the fact that they are known to target peak calcium current as opposed to LTCC inactivation.

Furthermore, despite that LCSD is known to be an effective treatment for patients with multiple arrhythmia syndromes,[36,37] every TKOS patient who underwent an LCSD experienced at least 1 post-LCSD BCE. However, the rate at which these patients experienced BCEs was reduced compared with drug therapy alone. Although the addition of a right cardiac sympathetic denervation could be an effective treatment strategy for some patients with TKOS, the current evidence is limited to fully support this.

This data makes clear the refractory nature of TKOS to conventional treatment and highlights the urgent need for novel therapies to treat this severe and potentially life-threatening disease. We surmise that a potential therapeutic option could be protein replacement using gene therapy. Because these patients are natural knockouts for triadin, TKOS serves as an ideal candidate for protein replacement treatment using

Table 4. Comparison of TKOS to Other Arrhythmia Syndromes

	Type	No. of Patients	QTc, ms	Age at First Event, y	Experienced Cardiac Event, %	Experienced Cardiac Arrest/SCD, %	Experienced BCE (%)	Ectopy on Stress Test (%)	Noncardiac Symptoms
LQTS									
Rohatgi et al 2017[15]	LQTS 1	287	460	11	22	1	4	…	None
	LQTS 2	204	466	14	32	2	8	…	None
	LQTS 3	56	474	0	23	4	20	…	None
Priori et al 2003[16]	LQTS 1	386	466	13	30	10	…	…	None
	LQTS 2	206	490	18	46	20	…	…	None
	LQTS 3	55	496	16	42	16	…	…	None
Zareba et al 1998[17]	LQTS 1	112	490	9	62	9	…	…	None
	LQTS 2	72	495	12	46	6	…	…	None
	LQTS 3	62	510	16	18	6	…	…	None
JLNS									
Schwartz et al 2006[13]		186	557	2	86	39	51	…	Sensorineural Deafness
TS									
Dufendach et al 2017[14]		17	640	…	…	65	…	…	Syndactyly, neurodevelopmental delay, facial abnormalities, baldness, hypoglycemia.
Calmodulinopathy									
Crotti et al 2016[18]		49	650	4	84	61	53	…	Neurodevelopmental Delay
TKOS									
Current Study		21	472	3	95	81	74	89	Skeletal Myopathy/ Muscle Weakness
CPVT									
Priori et al 2002[19]	CPVT1	19	…	8	57	30	37	74	None
Hayashi et al 2009[20]	CPVT1-2	101	403	12	69	24	23	87	None
Postma et al 2002[21]	CPVT2	3	424	8	100	33	33	100	None
di Barletta 2006[22]	CPVT2	2	…	4	100	0	50	100	None

BCE indicates breakthrough cardiac event; CPVT, catecholaminergic polymorphic ventricular tachycardia; JLNS, Jervell and Lange-Nielsen syndrome; LQTS, long QT syndrome; QTc, corrected QT interval; SCD, sudden cardiac death; TKOS, triadin knockout syndrome; and TS, Timothy syndrome.

adeno-associated virus serotype 9–based delivery of the normal cardiac triadin isoform.[38,39] Introducing normal cardiac triadin into the TKOS patient's cardiomyocytes could correct the abnormal arrhythmogenic phenotype and potentially cure the patient of their disease. In fact, this type of approach has previously been shown to be effective in treating recessively inherited, *CASQ2*-mediated CPVT2 in both mice and human patient-specific induced pluripotent stem cell-derived cardiomyocytes using adeno-associated virus serotype 9 delivery of WT-CASQ2.[40–42]

Therefore, as we seek to better care for patients experiencing this severe and treatment-refractory disease, we highly encourage their continued enrollment into the ITKOSR to better understand TKOS and to ready a well-characterized cohort for future *TRDN* gene therapy trials.

ARTICLE INFORMATION

Received October 16, 2018; accepted January 10, 2019.

The Data Supplement is available at https://www.ahajournals.org/doi/

suppl/10.1161/CIRCGEN.118.002419.

Correspondence

Michael J. Ackerman, MD, PhD, Mayo Clinic Windland Smith Rice Sudden Death Genomics Laboratory, Guggenheim 501, Mayo Clinic, 200 First St SW, Rochester, MN 55905. Email ackerman.michael@mayo.edu

Affiliations

Windland Smith Rice Sudden Death Genomics Laboratory, Department of Molecular Pharmacology and Experimental Therapeutics, Division of Pediatric Cardiology, Department of Pediatric and Adolescent Medicine, and Division of Heart Rhythm Services, Department of Cardiovascular Medicine, Mayo Clinic, Rochester, MN (D.J.C., D.J.T., J.R.G., J.M.B., R.K.R., M.J.A.). Department of Cardiology, Boston Children's Hospital and Harvard Medical School, MA (D.J.A.). Doernbecher Children's Hospital, Oregon Health and Science University, Portland (S.B.). Center for Cardiac Arrhythmias of Genetic Origin & Laboratory of Cardiovascular Genetics, Istituto Auxologico Italiano, IRCCS, Milan (L.C., P.J.S.). IRCCS Department of Cardiovascular, Neural & Metabolic Sciences, San Luca Hospital, Istituto Auxologico Italiano (L.C.). Department of Medicine and Surgery University of Milano-Bicocca, Italy (L.C.). Centre Hospitalier Universitaire de Grenoble Alpes (J.F., N.R.-B.). Institut des Neurosciences de Grenoble, INSERM U1216, Grenoble, France (J.F., N.R.-B.). Molecular Cardiology and Medicine Division, Istituti Clinici Scientifici Maugeri, IRCCS (C.N., S.G.P.). Department of Molecular Medicine, University of Pavia, Italy (C.N., S.G.P.). Reference Center for Rare Arrhythmic Disorders, Cardiologic Department, Nantes University Hospital, France (V.P.). L'institut du thorax, INSERM 1087, Nantes, France (V.P.). Electrophysiology and Heart Modeling Institute, Bordeaux University Hospital, IHU Liryc, University of Bordeaux, Pessac-Bordeaux, France (C.R.-T., F.S.). Children's Hospital Los Angeles, University of Southern California (M.J.S.). Paediatric Cardiology, University Hospital Bristol, United Kingdom (M.A.W.).

Sources of Funding

This work was supported by the Mayo Clinic Windland Smith Rice Comprehensive Sudden Cardiac Death Program.

Disclosures

Dr Ackerman is a consultant for Audentes Therapeutics, Boston Scientific, Gilead Sciences, Invitae, Medtronic, MyoKardia, and St. Jude Medical. Dr Ackerman and Mayo Clinic are involved in an equity/royalty relationship with AliveCor, Blue Ox Health Corporation, and Stemonix. Dr Balaji sits on an advisory board at yoR Labs and has received a research grant from Medtronic and consulting fees from MyoKardia. These relationships are all modest, and none of these entities have contributed to this study in any manner. The other authors report no conflicts.

REFERENCES

1. Kong MH, et al. Systematic review of the incidence of sudden cardiac death in the United States. *J Am Coll Cardiol*. 2011;57:794–801. doi: 10.1016/j.jacc.2010.09.064
2. Mehra R. Global public health problem of sudden cardiac death. *J Electrocardiol*. 2007;40(6 suppl):S118–S122. doi: 10.1016/j.jelectrocard.2007.06.023
3. Kung HC, et al. Deaths: final data for 2005. *Natl Vital Stat Rep*. 2008;56:1–120.
4. Roux-Buisson N, et al. Absence of triadin, a protein of the calcium release complex, is responsible for cardiac arrhythmia with sudden death in human. *Hum Mol Genet*. 2012;21:2759–2767. doi: 10.1093/hmg/dds104
5. Rooryck C, et al. New family with catecholaminergic polymorphic ventricular tachycardia linked to the triadin gene. *J Cardiovasc Electrophysiol*. 2015;26:1146–1150. doi: 10.1111/jce.12763
6. Walsh MA, et al. Compound heterozygous triadin mutation causing cardiac arrest in two siblings. *Pacing Clin Electrophysiol*. 2016;39:497–501. doi: 10.1111/pace.12813
7. O'Callaghan BM, et al. A unique triadin exon deletion causing a null phenotype. *HeartRhythm Case Rep*. 2018;4:514–518. doi: 10.1016/j.hrcr.2018.07.014
8. Altmann HM, et al. Homozygous/compound heterozygous triadin mutations associated with autosomal-recessive long-QT syndrome and pediatric sudden cardiac arrest: elucidation of the triadin knockout syndrome. *Circulation*. 2015;131:2051–2060. doi: 10.1161/CIRCULATIONAHA.115.015397
9. Moss AJ, et al. 25th anniversary of the International Long-QT Syndrome Registry: an ongoing quest to uncover the secrets of long-QT syndrome. *Circulation*. 2005;111:1199–1201. doi: 10.1161/01.CIR.0000157069.91834.DA
10. Guo W, et al. Biochemical characterization and molecular cloning of cardiac triadin. *J Biol Chem*. 1996;271:458–465.
11. Knollmann BC. New roles of calsequestrin and triadin in cardiac muscle. *J Physiol*. 2009;587(pt 13):3081–3087. doi: 10.1113/jphysiol.2009.172098
12. Chopra N, et al. Triadin regulates cardiac muscle couplon structure and microdomain Ca(2+) signalling: a path towards ventricular arrhythmias. *Cardiovasc Res*. 2013;98:187–191. doi: 10.1093/cvr/cvt023
13. Schwartz PJ, et al. The Jervell and Lange-Nielsen syndrome: natural history, molecular basis, and clinical outcome. *Circulation*. 2006;113:783–790. doi: 10.1161/CIRCULATIONAHA.105.592899
14. Dufendach KA, et al. Clinical outcomes and modes of death in timothy syndrome: a Multicenter International Study of a rare disorder. *JACC Clin Electrophysiol*. 2018;4:459–466. doi: 10.1016/j.jacep.2017.08.007
15. Rohatgi RK, et al. Contemporary outcomes in patients with long QT syndrome. *J Am Coll Cardiol*. 2017;70:453–462. doi: 10.1016/j.jacc.2017.05.046
16. Priori SG, et al. Risk stratification in the long-QT syndrome. *N Engl J Med*. 2003;348:1866–1874. doi: 10.1056/NEJMoa022147
17. Zareba W, et al. Influence of the genotype on the clinical course of the long-QT syndrome. International Long-QT Syndrome Registry Research Group. *N Engl J Med*. 1998;339:960–965. doi: 10.1056/NEJM199810013391404
18. Crotti L, et al. International Calmodulinopathy Regsitry (ICaMR). *Circulation*. 2016;134:A14840.
19. Priori SG, et al. Clinical and molecular characterization of patients with catecholaminergic polymorphic ventricular tachycardia. *Circulation*. 2002;106:69–74.
20. Hayashi M, et al. Incidence and risk factors of arrhythmic events in catecholaminergic polymorphic ventricular tachycardia. *Circulation*. 2009;119:2426–2434. doi: 10.1161/CIRCULATIONAHA.108.829267
21. Postma AV, et al. Absence of calsequestrin 2 causes severe forms of catecholaminergic polymorphic ventricular tachycardia. *Circ Res*. 2002;91:e21–e26.
22. di Barletta MR, et al. Clinical phenotype and functional characterization of CASQ2 mutations associated with catecholaminergic polymorphic ventricular tachycardia. *Circulation*. 2006;114:1012–1019. doi: 10.1161/CIRCULATIONAHA.106.623793
23. Nannenberg EA, et al. Effect of ascertainment bias on estimates of patient mortality in inherited cardiac diseases. *Circ Genom Precis Med*. 2018;11:e001797. doi: 10.1161/CIRCGEN.117.001797
24. Peng M, et al. Structural diversity of triadin in skeletal muscle and evidence of its existence in heart. *FEBS Lett*. 1994;348:17–20.
25. Kobayashi YM, et al. Identification of triadin 1 as the predominant triadin isoform expressed in mammalian myocardium. *J Biol Chem*. 1999;274:28660–28668.
26. Thevenon D, et al. Human skeletal muscle triadin: gene organization and cloning of the major isoform, Trisk 51. *Biochem Biophys Res Commun*. 2003;303:669–675.
27. Marty I. Triadin regulation of the ryanodine receptor complex. *J Physiol*. 2015;593:3261–3266. doi: 10.1113/jphysiol.2014.281147
28. Engel AG, et al. Congenital myopathy associated with the triadin knockout syndrome. *Neurology*. 2017;88:1153–1156. doi: 10.1212/WNL.0000000000003745
29. Dickinson DF. The normal ECG in childhood and adolescence. *Heart*. 2005;91:1626–1630. doi: 10.1136/hrt.2004.057307
30. Chopra N, et al. Ablation of triadin causes loss of cardiac Ca2+ release units, impaired excitation-contraction coupling, and cardiac arrhythmias. *Proc Natl Acad Sci USA*. 2009;106:7636–7641. doi: 10.1073/pnas.0902919106
31. Oddoux S, et al. Triadin deletion induces impaired skeletal muscle function. *J Biol Chem*. 2009;284:34918–34929. doi: 10.1074/jbc.M109.022442
32. Schwartz PJ, et al. The long QT syndrome: a transatlantic clinical approach to diagnosis and therapy. *Eur Heart J*. 2013;34:3109–3116. doi: 10.1093/eurheartj/eht089
33. van der Werf C, et al. Therapeutic approach for patients with catecholaminergic polymorphic ventricular tachycardia: state of the art and future developments. *Europace*. 2012;14:175–183. doi: 10.1093/europace/eur277

34. van der Werf C, et al. Flecainide therapy reduces exercise-induced ventricular arrhythmias in patients with catecholaminergic polymorphic ventricular tachycardia. *J Am Coll Cardiol*. 2011;57:2244–2254. doi: 10.1016/j.jacc.2011.01.026
35. Liu N, et al. Short communication: flecainide exerts an antiarrhythmic effect in a mouse model of catecholaminergic polymorphic ventricular tachycardia by increasing the threshold for triggered activity. *Circ Res*. 2011;109:291–295. doi: 10.1161/CIRCRESAHA.111.247338
36. Bos JM. Left cardiac sympathetic denervation in long QT syndrome: analysis of therapeutic nonresponders. *Circ Arrhythm Electrophysiol*. 2013;6:705–711. doi: 10.1161/CIRCEP.113.000102
37. De Ferrari GM, et al. Clinical management of catecholaminergic polymorphic ventricular tachycardia: the role of left cardiac sympathetic denervation. *Circulation*. 2015;131:2185–2193. doi: 10.1161/CIRCULATIONAHA.115.015731
38. Bongianino R, et al. Gene therapy to treat cardiac arrhythmias. *Nat Rev Cardiol*. 2015;12:531–546. doi: 10.1038/nrcardio.2015.61
39. Hammoudi N, et al. Adeno-associated virus-mediated gene therapy in cardiovascular disease. *Curr Opin Cardiol*. 2015;30:228–234. doi: 10.1097/HCO.0000000000000159
40. Denegri M, et al. Viral gene transfer rescues arrhythmogenic phenotype and ultrastructural abnormalities in adult calsequestrin-null mice with inherited arrhythmias. *Circ Res*. 2012;110:663–668. doi: 10.1161/CIRCRESAHA.111.263939
41. Denegri M, et al. Single delivery of an adeno-associated viral construct to transfer the CASQ2 gene to knock-in mice affected by catecholaminergic polymorphic ventricular tachycardia is able to cure the disease from birth to advanced age. *Circulation*. 2014;129:2673–2681. doi: 10.1161/CIRCULATIONAHA.113.006901
42. Lodola F, et al. Adeno-associated virus-mediated CASQ2 delivery rescues phenotypic alterations in a patient-specific model of recessive catecholaminergic polymorphic ventricular tachycardia. *Cell Death Dis*. 2016;7:e2393. doi: 10.1038/cddis.2016.304