Tzu Chi Medical Journal 2021; 33(4): 339-344

Review Article

Calmodulinopathy in inherited arrhythmia syndromes

*Wen-Chin Tsai[a], Peng-Sheng Chen[b,c], Michael Rubart[c,d]**

[a]Department of Cardiology, Cardiovascular Research Center, Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, and Tzu Chi University, Hualien, Taiwan, [b]Department of Cardiology, Cedar-Sinai Medical Center, Los Angeles, CA, USA, [c]Krannert Institute of Cardiology, Department of Medicine, Indiana University School of Medicine, Indianapolis, IN, USA, [d]Department of Pediatrics, Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, IN, USA

Submission : 21-Jul-2020
Revision : 02-Sep-2020
Acceptance : 07-Oct-2020
Web Publication : 14-Apr-2021

Abstract

Calmodulin (CaM) is a ubiquitous intracellular calcium sensor that controls and regulates key cellular functions. In all vertebrates, three CaM genes located on separate chromosomes encode an identical 149 amino acid protein, implying an extraordinarily high level of evolutionary importance and suggesting that CaM mutations would be possibly fatal. Inherited arrhythmia syndromes comprise a spectrum of primary electrical disorders caused by mutations in genes encoding ion channels or associated proteins leading to various cardiac arrhythmias, unexplained syncope, and sudden cardiac death. CaM mutations have emerged as an independent entity among inherited arrhythmia syndromes, referred to as calmodulinopathies. The most common clinical presentation associated with calmodulinopathy is congenital long QT syndrome, followed by catecholaminergic polymorphic ventricular tachycardia, both of which significantly increase the possibility of repeated syncope, lethal arrhythmic events, and sudden cardiac death, especially in young individuals. Here, we aim to give an overview of biochemical and structural characteristics of CaM and progress toward updating current known CaM mutations and associated clinical phenotypes. We also review the possible mechanisms underlying calmodulinopathy, based on several key *in vitro* studies. We expect that further experimental studies are needed to explore the complexity of calmodulinopathy.

Keywords: *Calmodulinopathy, Catecholaminergic polymorphic ventricular tachycardia, Long QT syndrome and inherited arrhythmia syndromes*

Introduction

Calcium ions (Ca^{2+}) influence nearly every aspect of cellular life. Calmodulin (CaM), a ubiquitously expressed Ca^{2+}-binding protein, has a vital role in relaying Ca^{2+} signals into biochemical and biomechanical responses by altering protein–protein interactions. As a result, CaM regulates a wide spectrum of cellular functions, including metabolism, gene expression, proliferation, contraction, and proteolysis [1]. No other molecule highlights the evolutionary importance of Ca^{2+} signaling. Although the properties and biological functions of CaM have been widely studied since its early discovery by Cheung in 1970, there are still considerable knowledge gaps that limit our understanding of Ca^{2+}/CaM interactions with and the identity of its target proteins [2].

Inherited arrhythmia syndromes are electrical abnormalities of the heart caused by mutations in genes encoding cardiac ion channels or associated proteins. Unfortunately, sudden cardiac death, an unexpected natural death, might be the first clinical presentation in patients with inherited arrhythmia syndrome, especially in young adolescence. Arrhythmia syndromes may manifest as catecholaminergic polymorphic ventricular tachycardia (CPVT) and/or long QT syndrome (LQTS). The first arrhythmogenic CaM mutation was identified in a large Swedish family with severe and inherited CPVT, causing syncope and sudden death [3]. Since then, more and more CaM mutations have been identified in patients, especially in young individuals, presenting with CPVT and/or LQTS.

Calmodulinopathy has emerged as another life-threatening, inherited arrhythmia syndrome. We summarize currently available knowledge on inherited arrhythmia syndromes. Specifically, we focus on this severe arrhythmogenic syndrome, termed calmodulinopathy, and also review CaM-encoding genes, protein structure and function, as well as the spectrum of CaM mutations and their associated phenotypes. Finally, we review the possible mechanisms underlying this complex arrhythmia syndrome.

Access this article online

Quick Response Code:

Website: www.tcmjmed.com

DOI: 10.4103/tcmj.tcmj_182_20

Address for correspondence:
Dr. Michael Rubart,
Department of Pediatrics, Wells Center for Pediatric Research, Indiana University School of Medicine, 1044 West Walnut Street, Indianapolis, IN 46202, USA.
E-mail: mrubartv@iu.edu

How to cite this article: Tsai WC, Chen PS, Rubart M. Calmodulinopathy in inherited arrhythmia syndromes. Tzu Chi Med J 2021; 33(4): 339-44.

Calmodulin genes, structure, and function

CaM is a small, ubiquitous adaptor protein that transduces Ca^{2+} signals into activities of downstream effector proteins. Three different CaM genes (*CALM1, CALM2,* and *CALM3*), located on three different chromosomes (14q32.11, 2p21, and 19q13.32, respectively), encode an identical, 149 amino acid protein. CaM consists of an N- and a C-lobe, connected by an α-helix linker. Each lobe contains two EF-hand motifs, each one binding one Ca^{2+} ion. Thus, CaM can bind up to 4 Ca^{2+} ions with dissociation constants (K_D) ranging from 1 to 10 μM [4]. Calcium binding changes CaM conformation, exposing a methionine-rich hydrophobic patch, at either N- or C-lobe, suitable for peptide binding sequences in its target molecules [5]. Intriguingly, the evolutionary significance of CaM is emphasized not only by the appearance of three different genes in three different chromosomes but also by the highly conserved protein sequence across all vertebrates [6]. CaM in both its Ca^{2+}-free (Apo-CaM) and Ca^{2+}-bound form (Ca^{2+}-CaM) significantly impacts on ion homeostasis of cardiomyocytes. Excitation contraction coupling is the process linking electrical excitation to contraction. Pacemaker cells undertake spontaneous depolarization and generate propagating action potentials, which lead to coordinated contraction of the heart. CaM modulates cardiac action potential through modulation of ion channel gating, including cardiac sodium, calcium, and potassium channels. Furthermore, CaM acting as an essential Ca^{2+} sensor interacts with multiple key downstream proteins by modulating Ca^{2+} influx, sarcoplasmic reticulum Ca^{2+} release, and Ca^{2+} recycling during cardiac excitation–contraction coupling [7]. Therefore, CaM gene mutations result in dysfunction of cardiac ion channels, leading to arrhythmias.

Inherited arrhythmia syndromes

Inherited arrhythmia syndromes manifest as long and short QT syndrome, Brugada syndrome, CPVT, and early repolarization syndrome [8]. Table 1 lists prevalence, electrocardiographic characteristics, underlying gene mutation, sex preference, age of onset, and known triggers for each type of congenital arrhythmia syndromes. The majority of inherited arrhythmia syndrome present with a disease-specific electrocardiogram (ECG) phenotype, demarcated by abnormal depolarization and/or repolarization, such as the coved-type ST-segment elevation in the right precordial leads, J-point elevation in inferolateral leads, and long QT and short QT intervals [Table 1]. These genetic abnormalities, implying altered imbalance in cardiac ion channel activities, provide an arrhythmogenic substrate that increases the risk of developing ventricular arrhythmias and subsequent sudden cardiac death, often in young individuals. With the recent advance in genetic testing, disease-causing genes for inherited arrhythmias have been successfully mapped, which has a great advantage in individualized patient care. Genetic testing in a proband or linkage mapping in affected family members is highly valuable in the diagnosis of inherited arrhythmia syndromes [8]. The LQTS is the best example for correlation between a certain mutation (genotype) and clinical presentation (phenotypes) [9]. CaM mutation-related inherited arrhythmias have been discovered in patients with a severe cardiac phenotype, characterized by a high penetrance, thus postulating a high propensity to lethal ventricular arrhythmias, syncope, and sudden cardiac death at a young age.

Reported inherited arrhythmia syndromes associated with calmodulinopathy

Inherited arrhythmia syndromes are found in more than half of unexplained cases of sudden cardiac death in young persons. Although the incidence is low, an early diagnosis helps initiating therapies to reduce the risk of sudden cardiac death. In 2012, Nyegaard and colleagues first identified two unrelated, *de novo* missense mutations (p. Asn54Ile; p. Asn98Ser) in the *CALM1* gene (start methionine denoted residue 1), leading to the development of early-onset CPVT or CPVT-like arrhythmia [3]. In 2013, Crotti *et al*. discovered three heterozygous *de novo* mutations in either *CALM1* (p. Asp130Gln; p. Phe142 Leu) or *CALM2* (p. Asp96Val) genes, presenting with recurrent cardiac arrest and dramatically prolonged QTc interval [19]. Since then, more mutations in the CaM-encoding genes *CALM1*, *CALM2,* and *CALM3* have been reported and linked to ventricular arrhythmias manifesting with features of CPVT and/or LQTS [19-28].

CPVT is an inherited arrhythmia syndrome, characterized by a normal resting ECG and polymorphic ventricular tachycardia induced by exercise or emotional stress in the absence of structural heart disease [13]. Patients typically experience episodes of syncope and even sudden cardiac death, especially in young individuals. If untreated, the disease is highly lethal, as approximately 30% of those affected experience at least one cardiac arrest and up to 80% one or more syncopal spells [14]. Heterozygosity for mutations in the ryanodine receptor 2 gene (RYR2 [MIM 180902], CPVT1) [29], or calsequestrin-2 gene (CASQ2 [MIM114251], CPVT2) [30] is known to cause the autosomal dominant inheritance pattern of CPVT. CALM1 and CALM2 mutations associated with CPVT were named CPVT4 [15]. Beta-blockers are considered first-line therapy, but unfortunately, they are not entirely effective in preventing life-threatening arrhythmias. A cardioverter–defibrillator is often implanted in patients who continue to have ventricular arrhythmias despite beta-blocker therapy. Other anti-arrhythmic agents, including flecainide and Ca^{2+} channel blockers, have also been used successfully in some cases. Small-scale studies show that left sympathetic ganglionectomy is effective in patients who are insufficiently protected by beta-blocker therapy and/or experience repeated electrical shocks from implanted defibrillators [31].

The congenital LQTS is a primary cardiac electrical disease, characterized by a prolongation of the QT interval on the ECG and by the manifestation of unexplained syncope or even cardiac arrest, mainly triggered by emotional or physical stress [9]. The QT interval reflects the duration of the ventricular action potential, which is determined by the function of different ion channels. Mutations in *KCNQ1* (LQT1, voltage-gated potassium channel), *KCNH2* (LQT2, voltage-gated potassium channel), and *SCN5A* (LQT3, voltage-gated sodium channel) account for at least 75% of congenital LQTS [10,24]. LQTSs caused by

Table 1: Summary of inherited arrhythmia syndromes

Arrhythmia	Prevalence	ECG characteristic	Gene/ion channel /function	Gender	Age of onset	Triggers of cardiac events
Long QT syndrome [9-12]	1/2000	12-lead ECG with Bazett's formula QTc ≥500 ms or QTc between 480 and 499 ms with unexplained syncope	KCNQ1 (30%- 35%), $\downarrow I_{Ks}$ KCNH2 (25%- 40%), $\downarrow I_{Kr}$ SCN5A (5%- 10%), $\uparrow I_{Na}$	Female>male	<20 years	LQT1: Exercise (swimming) LQT2: Acute arousal LQT3: Rest
Catecholaminergic polymorphic ventricular tachycardia [12-15]	1/10,000	Exercise or catecholamine-induced bidirectional VT or polymorphic ventricular premature beats or VT	RYR2 (60%- 65%), ↓ CASQ2 (3%- 5%), ↓	Same	<15	Adrenergic stimulus (exercise/emotional stress)
Brugada syndrome [12,16]	0.5- 1/1000 (South Asia)	ST elevation with type I morphology at lead V1 and V2, located at 2nd, 3rd, or 4th intercostal space spontaneously or after the challenge of class IC drugs	SCN5A (20%), $\uparrow I_{Na}$	Female/male: 1/5	30- 50 years	Fever, sleep
Early repolarization syndrome [12,17]	Unknown	J-point elevation ≥1 mm in ≥2 contiguous inferior and/ or lateral leads of a standard 12-lead ECG	CACNA1C (4%), ↓ICa CACNB2b (8%), $\downarrow I_{Ca}$ CACNA2D1 (4%), $\downarrow I_{Ca}$	Female/male: 1/4	30- 50	Short coupled premature beats
Short QT syndrome [12,18]	0.2- 1/1000	12-lead ECG with Bazett's formula QTc ≤330 ms	KCNH2 (<5%), $\uparrow I_{Kr}$ KCNQl (<5%), $\uparrow I_{Ks}$ KCNJ2 (<5%), $\uparrow I_{K1}$	Female/male: 1/5	Same	Unknown, mainly at rest

ECG: Electrocardiogram

mutations in *CALM1, 2,* and *3* genes are categorized as LQT 14, 15, and 16, respectively [11]. In symptomatic, untreated LQT patients, mortality is very high, reaching 21% within one year after the first syncopal episode [32]. In sharp contrast, with proper treatment, mortality is less than 1% during a 15-year follow-up [9], which makes the existence of symptomatic but undiagnosed patients unacceptable. Lifestyle modification, beta-blockers, left cardiac sympathetic denervation, and implantable cardioverter-defibrillator are the most important therapeutic modalities in the proper management of LQTS patients [31].

Table 2 lists arrhythmia syndromes associated with CaM mutations. Most patients develop symptoms in early life, even at the gestational stage. LQTS seems to be the dominant phenotype. By far, there is no overt sex or race prevalence. The functional consequences of the CaM mutations appear to be sufficiently severe to override any influence on the phenotype by the racial or gender background. Interestingly, carriers of the p. N98S, p. D132E, and p. Q132P CaM mutations displayed combined arrhythmia syndromes of CPVT and LQTS.

In line with the most extensive International Calmodulinopathy Registry [21], among 74 subjects carrying a pathogenic CaM mutation, 35 single-nucleotide substitutions were identified in the 74 CALM-positive patients (36 *CALM1*, 23 *CALM2*, and 15 *CALM3* patients) using whole-exome sequencing, targeted next-generation sequencing, or Sanger sequencing. These 35 variants occurred similarly among 3 CALM genes. Sixty-four (86.5%) were symptomatic, and the 10-year cumulative mortality was 27%. The predominant phenotype is LQTS (49%), followed by CPVT (28%). LQTS patients have high incidences of life-threatening arrhythmias (78%) with a median onset age of 1.5 years and poor response to therapies. The ECG pattern resembles that of LQTS type 3, which had a worse prognosis when compared to LQTS type 1 and type 2. All CPVT patients were symptomatic with a median onset age of 6.0 years, and 48% of which presented with major arrhythmic events. The registry discovered another combined phenotype, p. D134N. Undoubtedly, calmodulinopathy has become one of the main disease entities among patients with inherited arrhythmia syndromes.

Underlying mechanisms of calmodulinopathies

Dysregulation of Ca^{2+} entry into the cytosol of cardiomyocytes has been proposed as the primary mechanism of arrhythmias associated with CaM mutations, either resulting from increased Ca^{2+} entry through inactivation-incompetent L-type Ca^{2+} channels ($Ca_V1.2$) or from increased SR Ca^{2+} release via disinhibited cardiac ryanodine receptors (RyR2) [22,37,38].

Several *in vitro* studies have proven that *RyR2* and *CASQ2* gene mutations lead to an increased diastolic leakage of Ca^{2+} from the sarcoplasmic reticulum of ventricular cardiomyocytes, particularly under adrenergic stimulation, promoting delayed afterdepolarization-induced triggered activities [39,40]. CaM physiologically inhibits RyR2 activity. The CPVT-associated CaM mutations p. N54I, p. N98S, and p. A103V all showed RyR2 disinhibition and, therefore, augmented RyR2 opening probability leading to Ca^{2+} waves, which in turn may cause delayed afterdepolarizations [Table 3]. However, it has remained unclear whether CaM mutation-induced RyR2 dysfunction underlies CPVT *in vivo*.

Table 2: List of published calmodulin mutations-related inherited arrhythmia syndromes

Gene	Mutation	Phenotypes	Sex	Age of onset (years)	Race	Reference
CALM 1	p.N54I	CPVT	Male	12	White- Swedish	[3]
	p.F90L	IVF	Male	16	?	[33]
	p.N98S	CPVT; LQTS	Female; Male	4; 5	Iraqi; White?	[3,34]
	p.E105A	LQTS	Male	6	Japanese	[27]
	p.D130G	LQTS	Female; male	<1	White- Italy; Grecian	[19]
	p.D132V	LQTS	Male	3	White?	[35]
	p.E141G	LQTS	Male	4	Indian	[20]
	p.E141V	LQTS	Male	<1	Maltese	[28]
	p.F142L	LQTS	Female/Male	All <1	White, Black, Hispanic	[19,20]
CALM 2	p.D96V	LQTS	Female	<1	Hispanic	[19]
	p.N98S	LQTS/CPVT	Male/Female	5/4	Japanese/Moroccan; Hispanic	[23,25]
		LQTS+CPVT	Male	7		
	p.N98I	LQTS	Male	17	Japanese	[25]
	p.D130G	LQTS	Female	<1	Indian	[20]
	p.D130V	LQTS	Male	<1	White	[20]
	p.D132E	LQTS+CPVT	Female	<9	White-Germany	[25]
	p.D132H	LQTS	Male	<1	White?	[35]
	p.D134H	LQTS	Female	<1	Japanese	[25]
	p.Q136P	LQTS+CPVT	Female	8	Morracan	[25]
CALM 3	p.D94A	LQTS	Female	8	White	[34]
	p.D96H	LQTS	Female	<1	White?	[36]
	p. A103V	CPVT	Female	10	White?	[22]
	p.D130G	LQTS	Male; female	<1	White	[26,28]
	p.E141K	LQTS	Female; male	<1	Hispanic? Saudi Arabia	[28]
	p.F142L	LQTS	Female	<1	White?	[36]

CPVT: Catecholaminergic polymorphic ventricular tachycardia, LQTS: Long QT syndrome, IVF: Idiopathic ventricular fibrillation

Table 3: The biophysical effects of reported calmodulinopathy

CaM mutation	Gene	Phenotype	Location of mutation	CaM-C Domain Kd (Fold Reduction)	I_{Ca}, CDI	APD	Ca^{2+}spark	Binding to RyR2	Inhibition of RyR2
N54I [22,37,38]	*CALM1*	CPVT	EF hand I-II linker	−	−	−	↑	↑ at low [Ca^{2+}] − at high [Ca^{2+}]	↓at low and high [Ca^{2+}]
D96V [22,37,38,41]	*CALM2*	LQTS	EF hand III	13.6	Loss	↑	−	− at low [Ca^{2+}] ↑ at high [Ca^{2+}]	− at low and high [Ca^{2+}]
N98I [25]	*CALM2*	LQTS	EF hand III	8.3	Nil	Nil	Nil	Nil	Nil
N98S [23,38,42]	*CALM1/2*	CPVT/LQTS CPVT+LQTS	EF hand III	3.3	Impaired	↑	↑	↑ at low [Ca^{2+}] − at high [Ca^{2+}]	− at low [Ca^{2+}] ↓at high [Ca^{2+}]
A103V [22]	*CALM3*	CPVT	EF hand III	2.9	−	−	↑	−	↓at low [Ca^{2+}]
D130G [37,38,41,43]	*CALM1/2/3*	LQTS	EF hand IV	53.6	Loss	↑	↓	↓at low and high [Ca^{2+}]	↑at low [Ca^{2+}]
D132E [25]	*CALM2*	CPVT+LQTS	EF hand IV	22.9	Nil	Nil	Nil	Nil	Nil
D132H [35]	*CALM2*	LQTS	EF hand IV	77.0	Impaired	Nil	Nil	Nil	Nil
D132V [35]	*CALM1*	LQTS	EF hand IV	63.5	Impaired	Nil	Nil	Nil	Nil
D134H [25]	*CALM2*	LQTS	EF hand IV	12.9	Nil	Nil	Nil	Nil	Nil
Q136P [25]	*CALM2*	CPVT+LQTS	EF hand IV	6.5	Nil	Nil	Nil	Nil	Nil
E141G [44]	*CALM1*	LQTS	C terminal	10.8	Loss	Nil	Nil	Nil	− at low [Ca^{2+}]
E141K [28]	*CALM3*	LQTS	C terminal	32.6	Nil	Nil	Nil	Nil	Nil
E141V [28]	*CALM1*	LQTS	C terminal	24.7	Nil	Nil	Nil	Nil	Nil
F142L [37,38,45]	*CALM1/3*	LQTS	C terminal	5.4	Loss	↑	↓	↓at low [Ca^{2+}] − at high [Ca^{2+}]	↑at low [Ca^{2+}]

CDI: Ca^{2+}-dependent inactivation, CPVT: Catecholaminergic polymorphic ventricular tachycardia, LQTS: Long QT syndrome, APD: Action potential duration, EF: Ejection fraction

The properties of $Ca_V1.2$ control cardiac action potential generation, morphology, and duration. Furthermore, $Ca_V1.2$ plays the primary role in providing Ca^{2+} for the initiation of Ca^{2+}-induced Ca^{2+} release in cardiac myocytes. During the repolarization phase, Ca^{2+}-dependent inactivation (CDI) of $Ca_V1.2$ limits Ca^{2+} entry under physiological conditions, and disruption of this vital feedback mechanism is known to result in severe LQTS. For example, LQTS 8, known as Timothy syndrome, is associated with severe defects in CDI [46]. Correspondingly, loss of CDI was observed for several LQTS-causing CaM mutations, including p. D96V, p. D130G, p. E141G, and p. F142 L. So far, all patients with calmodulinopathy only harbor a mutation in 1 out of 6 CaM-encoding alleles. Taking advantages of using induced pluripotent stem cell-derived cardiomyocytes from LQTS patients heterozygous for mutations in one CaM gene (*CALM1*-F142 L; *CALM2*-N98S/D130G), several recent studies have demonstrated a strong dominant-negative effect of single-mutant CaM alleles on CDI of $Ca_V1.2$, resulting in prolonged action potential duration, which could be rescued by selectively knocking out the mutant gene while sparing the wild-type counterparts [42,43,45].

The detailed mechanisms of how a mutation in one of the six alleles leads to such severe arrhythmia syndromes remain unclear. There is a complex genotype–phenotype correlation. Generally speaking, the magnitude of Ca^{2+} affinity reduction correlated with the severity of CDI impairment of $Ca_V1.2$, which in turn would decide the amplitude of action potential prolongation and believed to be a major cause of LQTS. On the other hand, dysfunctional RyR-mediated Ca^{2+} handling has been implicated in the pathogenesis of cardiac arrhythmias, especially in established CPVT mouse model and documented human CPVT. Ca^{2+} leak through RyR can cause triggered activity in the form of delayed afterdepolarizations, which have the propensity to evoke a premature beat to initiate the arrhythmia. In addition, *in vitro* studies had proved that Purkinje cells are critical contributors to arrhythmic triggers in established mouse models of CPVT, which might implicate the possible role of Purkinje fiber in the calmodulinopathy [47,48]. However, the effect of CaM mutation on the function RyR2 is more complex and not directly linked to Ca^{2+} affinity. For example, the phosphorylation of RyR2 and subsequent sarcoplasmic reticulum Ca^{2+} release is dependent on Ca^{2+}/CaM-dependent kinase II, which adds an additional layer of complexity when trying to understanding the pathogenesis. As for those carriers, displaying the phenotypes of either CPVT, LQTS, or both, environmental factors or modifier genes might also have an influence on the clinical manifestation.

Conclusion

Since the first case of CaM mutation-associated CPVT was identified in 2012, calmodulinopathies have emerged as a novel cause of human inherited arrhythmia syndromes. Thus far, approximately 30 CaM mutations with clear disease association have been recognized. A better understanding of the fundamental mechanisms is mandatory. Several *in vitro* biochemical, as well as cellular studies, have demonstrated that particularly, the regulation of the $Ca_V1.2$ and RyR2 is affected by these mutations. We have developed the first knock-in mouse model, heterozygous for the p. N98S mutation in *Calm1*, which presents with an overlap of CPVT and LQTS [49,50]. Future studies using this novel mouse model should have a great value for unraveling calmodulinopathy mechanisms as well as the possible treatment options for patients suffering from CaM-associated inherited arrhythmia syndromes.

Financial support and sponsorship

The work was supported by the grant of Buddhist Tzu Chi Medical Foundation (TCMMP 109-01).

Conflicts of interest

There are no conflicts of interest.

References

1. Sharma RK, Parameswaran S. Calmodulin-binding proteins: A journey of 40 years. Cell Calcium 2018;75:89-100.
2. Cheung WY. Cyclic 3',5'-nucleotide phosphodiesterase. Demonstration of an activator. Biochem Biophys Res Commun 1970;38:533-8.
3. Nyegaard M, Overgaard MT, Søndergaard MT, Vranas M, Behr ER, Hildebrandt LL, et al. Mutations in calmodulin cause ventricular tachycardia and sudden cardiac death. Am J Hum Genet 2012;91:703-12.
4. Linse S, Helmersson A, Forsén S. Calcium binding to calmodulin and its globular domains. J Biol Chem 1991;266:8050-4.
5. Chou JJ, Li S, Klee CB, Bax A. Solution structure of Ca(2+)-calmodulin reveals flexible hand-like properties of its domains. Nat Struct Biol 2001;8:990-7.
6. Yamniuk AP, Vogel HJ. Calmodulin's flexibility allows for promiscuity in its interactions with target proteins and peptides. Mol Biotechnol 2004;27:33-57.
7. Sorensen AB, Sondergaard MT, Overgaard MT. Calmodulin in a heartbeat. FEBS J 2013;280:5511-32.
8. Offerhaus JA, Bezzina CR, Wilde AAM. Epidemiology of inherited arrhythmias. Nat Rev Cardiol 2020;17:205-15.
9. Schwartz PJ, Crotti L, Insolia R. Long-QT syndrome: From genetics to management. Circ Arrhythm Electrophysiol 2012;5:868-77.
10. Tester DJ, Ackerman MJ. Genetics of long QT syndrome. Methodist Debakey Cardiovasc J 2014;10:29-33.
11. Giudicessi JR, Wilde AAM, Ackerman MJ. The genetic architecture of long QT syndrome: A critical reappraisal. Trends Cardiovasc Med 2018;28:453-64.
12. Priori SG, Wilde AA, Horie M, Cho Y, Behr ER, Berul C, et al. HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes: Document endorsed by HRS, EHRA, and APHRS in May 2013 and by ACCF, AHA, PACES, and AEPC in June 2013. Heart Rhythm 2013;10:1932-63.
13. Leenhardt A, Lucet V, Denjoy I, Grau F, Ngoc DD, Coumel P. Catecholaminergic polymorphic ventricular tachycardia in children. A 7-year follow-up of 21 patients. Circulation 1995;91:1512-9.
14. Napolitano C, Priori SG, Bloise R. Catecholaminergic polymorphic ventricular tachycardia. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, *et al*., editors. GeneReviews((R)), University of Washington, Seattle; 1993.
15. Lieve KV, van der Werf C, Wilde AA. Catecholaminergic Polymorphic Ventricular Tachycardia. Circ J 2016;80:1285-91.
16. Obeyesekere MN, Klein GJ, Modi S, Leong-Sit P, Gula LJ, Yee R, et al. How to perform and interpret provocative testing for the diagnosis of Brugada syndrome, long-QT syndrome, and catecholaminergic polymorphic ventricular tachycardia. Circ Arrhythm Electrophysiol

2011;4:958-64.

17. Dalos D, Fiedler L, Radojevic J, Sponder M, Dichtl W, Schukro C. Prevalence of early repolarization syndrome and long-term clinical outcome in patients with the diagnosis of idiopathic ventricular fibrillation. Heart Vessels 2019;34:625-31.
18. Guerrier K, Kwiatkowski D, Czosek RJ, Spar DS, Anderson JB, Knilans TK. Short QT interval prevalence and clinical outcomes in a pediatric population. Circ Arrhythm Electrophysiol 2015;8:1460-4.
19. Crotti L, Johnson CN, Graf E, De Ferrari GM, Cuneo BF, Ovadia M, et al. Calmodulin mutations associated with recurrent cardiac arrest in infants. Circulation 2013;127:1009-17.
20. Boczek NJ, Gomez-Hurtado N, Ye D, Calvert ML, Tester DJ, Kryshtal D, et al. Spectrum and prevalence of CALM1-, CALM2-, and CALM3-encoded calmodulin variants in long QT Syndrome and functional characterization of a novel long QT Syndrome-associated calmodulin missense variant, E141G. Circ Cardiovasc Genet 2016;9:136-46.
21. Crotti L, Spazzolini C, Tester DJ, Ghidoni A, Baruteau AE, Beckmann BM, et al. Calmodulin mutations and life-threatening cardiac arrhythmias: Insights from the International Calmodulinopathy Registry. Eur Heart J 2019;40:2964-75.
22. Gomez-Hurtado N, Boczek NJ, Kryshtal DO, Johnson CN, Sun J, Nitu FR, *et al*. Novel CPVT-associated calmodulin mutation in CALM3 (CALM3-A103V) activates arrhythmogenic Ca waves and sparks. Circ Arrhythm Electrophysiol 2016;9:10.1161.
23. Jimenez-Jaimez J, Palomino Doza J, Ortega A, Macias-Ruiz R, Perin F, Rodriguez-Vazquez del Rey MM, et al. Calmodulin 2 mutation N98S i aAssociated with unexplained crdiac Aarrest in infants due to low clinical penetrance electrical disorders. PLoS One 2016;11:e0153851.
24. Kapplinger JD, Tester DJ, Salisbury BA, Carr JL, Harris-Kerr C, Pollevick GD, et al. Spectrum and prevalence of mutations from the first 2,500 consecutive unrelated patients referred for the FAMILION long QT syndrome genetic test. Heart Rhythm 2009;6:1297-303.
25. Makita N, Yagihara N, Crotti L, Johnson CN, Beckmann BM, Roh MS, et al. Novel calmodulin mutations associated with congenital arrhythmia susceptibility. Circ Cardiovasc Genet 2014;7:466-74.
26. Reed GJ, Boczek NJ, Etheridge SP, Ackerman MJ. CALM3 mutation associated with long QT syndrome. Heart Rhythm 2015;12:419-22.
27. Takahashi K, Ishikawa T, Makita N, Takefuta K, Nabeshima T, Nakayashiro M. A novel *de novo* calmodulin mutation in a 6-year-old boy who experienced an aborted cardiac arrest. HeartRhythm Case Rep 2017;3:69-72.
28. Wren LM, Jiménez-Jáimez J, Al-Ghamdi S, Al-Aama JY, Bdeir A, Al-Hassnan ZN, et al. Genetic Mosaicism in Calmodulinopathy. Circ Genom Precis Med 2019;12:375-85.
29. Priori SG, Napolitano C, Tiso N, Memmi M, Vignati G, Bloise R, et al. Mutations in the cardiac ryanodine receptor gene (hRyR2) underlie catecholaminergic polymorphic ventricular tachycardia. Circulation 2001;103:196-200.
30. Lahat H, Pras E, Olender T, Avidan N, Ben-Asher E, Man O, et al. A missense mutation in a highly conserved region of CASQ2 is associated with autosomal recessive catecholamine-induced polymorphic ventricular tachycardia in Bedouin families from Israel. Am J Hum Genet 2001;69:1378-84.
31. Priori SG, Blomstrom-Lundqvist C, Mazzanti A, Blom N, Borggrefe M, Camm J, et al. 2015 ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: The task force for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death of the European Society of Cardiology (ESC). Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC). Eur Heart J 2015;36:2793-867.
32. Schwartz PJ. Idiopathic long QT syndrome: Progress and questions. Am Heart J 1985;109:399-411.
33. Marsman RF, Barc J, Beekman L, Alders M, Dooijes D, van den Wijngaard A, et al. A mutation in CALM1 encoding calmodulin in familial idiopathic ventricular fibrillation in childhood and adolescence. J Am Coll Cardiol 2014;63:259-66.
34. Daly A, Johnson NM, Decker E, Callis TE, Tahiliani J, Garcia J, et al. Pathogenic variants in calmodulin associated with resuscitated childhood cardiac arrest (Abstract C-AB01-05). Heart Rhythm 2017;14(Suppl 5):S2.
35. Pipilas DC, Johnson CN, Webster G, Schlaepfer J, Fellmann F, Sekarski N, et al. Novel calmodulin mutations associated with congenital long QT syndrome affect calcium current in human cardiomyocytes. Heart Rhythm 2016;13:2012-9.
36. Chaix MA, Koopmann TT, Goyette P, Alikashani A, Latour F, Fatah M, et al. Novel CALM3 mutations in pediatric long QT syndrome patients support a CALM3-specific calmodulinopathy. HeartRhythm Case Rep 2016;2:250-4.
37. Limpitikul WB, Dick IE, Joshi-Mukherjee R, Overgaard MT, George AL Jr., Yue DT. Calmodulin mutations associated with long QT syndrome prevent inactivation of cardiac L-type Ca(2+) currents and promote proarrhythmic behavior in ventricular myocytes. J Mol Cell Cardiol 2014;74:115-24.
38. Hwang HS, Nitu FR, Yang Y, Walweel K, Pereira L, Johnson CN, et al. Divergent regulation of ryanodine receptor 2 calcium release channels by arrhythmogenic human calmodulin missense mutants. Circ Res 2014;114:1114-24.
39. Cerrone M, Colombi B, Santoro M, di Barletta MR, Scelsi M, Villani L, et al. Bidirectional ventricular tachycardia and fibrillation elicited in a knock-in mouse model carrier of a mutation in the cardiac ryanodine receptor. Circ Res 2005;96:e77-82.
40. Knollmann BC, Chopra N, Hlaing T, Akin B, Yang T, Ettensohn K, et al. Casq2 deletion causes sarcoplasmic reticulum volume increase, premature Ca2+ release, and catecholaminergic polymorphic ventricular tachycardia. J Clin Invest 2006;116:2510-20.
41. Yin G, Hassan F, Haroun AR, Murphy LL, Crotti L, Schwartz PJ, et al. Arrhythmogenic calmodulin mutations disrupt intracellular cardiomyocyte Ca2+ regulation by distinct mechanisms. J Am Heart Assoc 2014;3:e000996.
42. Yamamoto Y, Makiyama T, Harita T, Sasaki K, Wuriyanghai Y, Hayano M, et al. Allele-specific ablation rescues electrophysiological abnormalities in a human iPS cell model of long-QT syndrome with a CALM2 mutation. Hum Mol Genet 2017;26:1670-7.
43. Limpitikul WB, Dick IE, Tester DJ, Boczek NJ, Limphong P, Yang W, et al. A precision medicine approach to the rescue of function on malignant calmodulinopathic long-QT syndrome. Circ Res 2017;120:39-48.
44. Søndergaard MT, Sorensen AB, Skov LL, Kjaer-Sorensen K, Bauer MC, Nyegaard M, et al. Calmodulin mutations causing catecholaminergic polymorphic ventricular tachycardia confer opposing functional and biophysical molecular changes. FEBS J 2015;282:803-16.
45. Rocchetti M, Sala L, Dreizehnter L, Crotti L, Sinnecker D, Mura M, et al. Elucidating arrhythmogenic mechanisms of long-QT syndrome CALM1-F142 L mutation in patient-specific induced pluripotent stem cell-derived cardiomyocytes. Cardiovasc Res 2017;113:531-41.
46. Dick IE, Joshi-Mukherjee R, Yang W, Yue DT. Arrhythmogenesis in Timothy Syndrome is associated with defects in Ca(2+)-dependent inactivation. Nat Commun 2016;7:10370.
47. Herron TJ, Milstein ML, Anumonwo J, Priori SG, Jalife J. Purkinje cell calcium dysregulation is the cellular mechanism that underlies catecholaminergic polymorphic ventricular tachycardia. Heart Rhythm 2010;7:1122-8.
48. Kang G, Giovannone SF, Liu N, Liu FY, Zhang J, Priori SG, et al. Purkinje cells from RyR2 mutant mice are highly arrhythmogenic but responsive to targeted therapy. Circ Res 2010;107:512-9.
49. Tsai WC, ET, Olaopa M, Field LJ, Chen PS, Rubart M. Mice heterozygous for the N98S mutation in Calm1 exhibit sinus bradycardia, long QTc interval, and catecholaminergic polymorphic ventricular tachycardia. Am Heart Assoc 2016; May (abstract).
50. Tsai WC, Guo S, Olaopa MA, Field LJ, Yang J, Shen C, *et al*. Complex arrhythmia syndrome in a knock-in mouse model carrier of the N98S Calm1 mutation. Circulation 2020;142:1937-55.