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Review Article

The link between abnormalities of calcium handling proteins and catecholaminergic polymorphic ventricular tachycardia

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Abstract

Catecholaminergic polymorphic ventricular tachycardia (CPVT), a rare autosomal dominant or recessive disease, usually results in syncope or sudden cardiac death. Most CPVT patients do not show abnormal cardiac structure and electrocardiogram features and symptoms, usually onset during adrenergically mediated physiological conditions. CPVT tends to occur at a younger age and is not easy to be diagnosed and managed. The main cause of CPVT is associated with mishandling Ca^{2+} in cardiomyocytes. Intracellular Ca^{2+} is strictly controlled by a protein located in the sarcoplasm reticulum (SR), such as ryanodine receptor, histidine-rich Ca^{2+}-binding protein, triadin, and junctin. Mutation in these proteins results in misfolding or malfunction of these proteins, thereby affecting their Ca^{2+}-binding affinity, and subsequently disturbs Ca^{2+} homeostasis during excitation–contraction coupling (E-C coupling). Furthermore, transient disturbance of Ca^{2+} homeostasis increases membrane potential and causes Ca^{2+} store overload-induced Ca^{2+} release, which in turn leads to delayed after depolarization and arrhythmia. Previous studies have focused on the interaction between ryanodine receptors and protein kinase or phosphatase in the cytosol. However, recent studies showed the regulation signaling for ryanodine receptor not only from the cytosol but also within the SR. The changing of Ca^{2+} concentration is critical for protein interaction inside the SR which changes protein conformation to regulate the open probability of ryanodine receptors. Thus, it influences the threshold of Ca^{2+} released from the SR, making it easier to release Ca^{2+} during E-C coupling. In this review, we briefly discuss how Ca^{2+} handling protein variations affect the Ca^{2+} handling in CPVT.

Keywords: *Ca^{2+} mishandling, Calsequestrin 2, Catecholaminergic polymorphic ventricular tachycardia, Ryanodine receptors 2, Triadin*

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Introduction

Catecholaminergic polymorphic ventricular tachycardia (CPVT), which is known as an electrical disorder, is characterized as the normal cardiac structure with normal rest electrocardiography (EKG), but drastic changes in EKG during exercise or emotion, or increases of stress. CPVT can lead to episodic syncope and sudden cardiac death [1,2]. It has been estimated that approximately 1/10,000 people has CPVT, which is usually diagnosed in the early stage of life with an average between 2 and 21 years (usually present between 7 and 11 years) [3,4]. The incidence of inheriting CPVT is around 30% of total patients, and it is in favor of female inheritance [3]. The majority of them are autosomal dominant (*RyR2*, *CALM*), and few of them are autosomal recessive (*CASQ2*, *TECRL*, and *TRDN*) [3,4]. The severity of symptoms in CPVT patients varies from only vasovagal syncope or long QT syndrome (LQTs) with normal QT interval (QTc) to severe arrhythmia and seizure [5,6]. Atypical cases have also been reported that some patients suffer from malignant hyperthermia, paroxysmal atrial fibrillation (AF), or sudden cardiac death during sleep [5-7].

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Diagnosis of catecholaminergic polymorphic ventricular tachycardia

It has been reported that CPVT can be diagnosed with various methods such as 12-lead EKG, exercise stress test, Holter monitoring, echocardiogram (for excluding structural defect), medical genetics consultation, and catecholamine or isoproterenol infusion [3,8]. The EKG features captured

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on 12-lead EKG during exercise or emotional test include mono- or polymorphic couplets, nonsustained ventricular tachycardia (VT), premature ventricular contractions, polymorphic VT, polymorphic or bidirectional VT, and monomorphic ventricular extrasystoles with episodes of bigeminus [3,9,10]. Although the sensitivity of Holter monitor is lower than EKG, it is suitable for patients who are unable to endure adequate exercise stress [10]. The exercise stress test is the most recommended diagnostic method for CPVT [4]. It has been indicated that when heartbeat reaches around 100–120 beats/min in CPVT patients during programmed ventricular stimulation, VT, polymorphic ventricular arrhythmia, and multifocal premature ventricular contractions will be induced, polymorphic VT is less common to be induced [9]. The majority of children with CPVT have bradycardia (≤60 bpm) compared with the healthy children (around 80–100 bpm) [11]. However, if the patients or their family member experienced unexplainable adrenergic triggering syncope events, implantable loop recorders and exercise stress test on a full dose of beta-blockers should be recommended [12,13]. The genetic test is recommended for the patient and the patient's family members who have been diagnosed with LQTs or suspected of having CPVT due to numerous reports associated with misdiagnosis of CPVT as LQTs. Letsas *et al*. have reported that both a 16-year-old female and her mother were previously diagnosed with LQTs and treated with implantable cardioverter-defibrillator. After the genetic test, the results showed that both of them were misdiagnosed [14]. Similar to LQTs, there are many diseases including Andersen-Tawil syndrome and arrhythmogenic right ventricular cardiomyopathy, present with similar feature of CPVT. Similar to LQTs, many diseases, including Andersen-Tawil syndrome and arrhythmogenic right ventricular cardiomyopathy, present similar feature of CPVT. Using mutational analysis, it is impossible to distinguish these three diseases, mainly because they have overlapping gene mutations. Thus, it is important to differentially diagnose them from CPVT when receiving young episodic syncope or arrhythmia patients [3,15,16].

Intracellular calcium handling dysfunction and catecholaminergic polymorphic ventricular tachycardia

In cardiomyocytes, calcium ions (Ca^{2+}) are mainly involved in the formation of excitation–contraction coupling (E-C coupling), which is initiated by a mechanism known as Ca^{2+}-induced Ca^{2+} release. During membrane depolarizing, the action potential (AP) activates the voltage-dependent L-type Ca^{2+} channel ($Ca_V1.2$) in the transverse tubular (T-tubules) membrane, leading to a small Ca^{2+} influx [17], which in turn activates ryanodine receptor 2 (RyR2) on the sarcoplasmic reticulum (SR) membrane, thereby causing Ca^{2+} to be released into the cytosol. The released Ca^{2+} binds to troponin C, subsequently causing a cascade of conformational changes in the myofilaments, and ultimately induces muscle contraction [17]. During the relaxation phase, the released Ca^{2+} is pumped back to the SR by the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) or extruded to the extracellular space by the Na^+/Ca^{2+} exchanger (NCX). This event effectively lowers the cytosolic Ca^{2+} concentration and allows Ca^{2+} dislocated from the myofilaments. The mishandling of Ca^{2+} during E-C coupling can cause Ca^{2+} store overload. The overload of the SR may cause spontaneous Ca^{2+} release after repolarization, which is termed store overload-induced Ca^{2+} release. The released Ca^{2+} exits the cell through the $3Na^+/Ca^{2+}$-exchanger, subsequently leading to delayed after depolarizations (DADs) [17-19]. There are numerous proteins related to Ca^{2+} handlings, such as RyR2, phospholamban (PLB or PLN), SERCA, calsequestrin 2 (CSQ2), L-type Ca^{2+} channels, NCX, calmodulin (CaM), Ca^{2+}/CaM-dependent protein kinase II (CaMKII), protein kinase A (PKA), triadin, junctin, ankyrin-B, and TECRL [17,20,21]. It is believed that mutation or malfunction in these proteins can cause Ca^{2+} release from the SR prematurely or spontaneously, hence inducing both atrial arrhythmia and VA [22,23]. The malfunction of these proteins is mostly related to the genetic mutation. These genes include *RYR2* for RYR2, *CASQ2* for CSQ2, *CALM1*, *CALM2*, and *CALM3* for CaM, *JCN* for junctin, *ANK2* for ankyrin B, *TECRL* for trans-2,3-enoyl-CoA reductase-like, and *TRDN* for triadin [21]. *RyR2* and *CASQ2* mutations account for the majority of CPVT patients which can be categorized in CPVT type 1 and CPVT type 2. *RyR2* mutation alone accounts for 60% of cases [24,25]. It has been reported that a small number of patients carrying *RYR2* gene mutations displayed a LQTs phenotype or an overlapping phenotype of LQTs and CPVT [26,27]. Further, mutation of *TECRL* can cause CPVT type 3, which presents a mixed phenotype of CPVT and LQTs [27]. CPVT types 4 and 5 are considered as minority and account for <1% of CPVT cases [3]. CPVT type 4, the result of *ANK2* and *CALM* gene mutations, starts around the age of 4 and has about 18% of sudden cardiac death. Mutation in the *ANK2* gene causes the ankyrin-B syndrome, which presents with long QT interval and impaired conduction of electrical impulses between chambers or even heart block. CPVT type 5, the result of *TRDN* gene mutation, starts around the age of 2–3 and has 25% of sudden cardiac death [3].

Catecholaminergic polymorphic ventricular tachycardia-associated ryanodine receptor mutations

Ryanodine receptors, which are Ca^{2+}-release channels located on the ER and SR membranes, contain three isoforms: RyR1, RyR2, and RyR3. RyR2 is the most important Ca^{2+} channel on the cardiac SR and plays a critical role in E-C coupling [28,29]. The activation of RyR2 depends on the phosphorylation of serine-2808 by CaMKII and serine-2030 by PKA [30]. It has also been suggested that serine-2811 might be the potential spot for CaMKII phosphorylation [31]. It has been reported that phosphorylation of RyR2 by both PKA and CaMKII increases the open probability and further releases Ca^{2+} from the SR [32-34]. Using the cardiomyocytes derived from CPVT patient-specifically induced pluripotent stem cells (iPSCs), Pölönen *et al*. discovered that patients carrying exon 3 deletions (E3D) and RYR2-p.L4115F increase the amount of DADs and early after depolarizations (EADs)

after adrenaline exposure. E3D-CPVT hiPSC-CMs have the shortest AP duration, lowest AP amplitude, upstroke velocity, and more depolarized diastolic potential than control. Further, E3D-CPVT hiPSC-CMs have an increased amount of DADs, EADs, and tachyarrhythmia [35]. Using cardiac tissue isolated from RyR2 P2328S homozygous mice, Salvage *et al*. discovered that this type of mutation increases the sensitivity to Ca^{2+} and reduces both the activation and inactivation threshold. Their discovery suggests that RyR2 P2328S homozygous mutation increases the total release of Ca^{2+} from the SR, causing AF and VA [36]. Using the RyR2 R4496C mouse model of CPVT crossing with the $C{\times}40^{EGFP/+}$ transgenic mouse, Herron *et al*. discovered that variation of RyR2 R4496C effects on Ca^{2+} homeostasis not only in ventricular myocytes but also in cardiac Purkinje cells [37]. Gallegos-Cortez *et al*. reported that a 12-year-old girl suffering from multiple falls while cycling was finally diagnosed with CPVT. She did not have any family history of syncope or sudden cardiac death. The genetic test showed that she carries a missense RYR2 mutation (p.Gly3946Ser) in a heterozygous state [9]. This kind of mutation has been predicted as pathogenic in the ClinVar database and reported in other patients [9,38]. Tung *et al*. analyzed a 108-member proband and reported three of the member's variation in the RYR2 gene (c. 527G>T, p.R176L). The RyR2 R176L increases DADs causing CPVT-associated sudden cardiac arrest [39].

Catecholaminergic polymorphic ventricular tachycardia-associated calsequestrin (CASQ) mutations

CASQ, a 399 amino acid protein, binds 40–50 Ca^{2+} and serves as a high capacity but low affinity Ca^{2+} buffering protein [40,41]. CASQ has two isoforms: CASQ1 and CASQ2, encoded by two different genes, *CASQ1* and *CASQ2*. CASQ2 can be found in both cardiac and slow-twitch skeletal muscle [41]. CASQ2 localizes to the junctional SR of the muscle and forms multimers, which are anchored to RyR2 by triadin and junctin on the luminal SR membrane [42]. Using the cardiac SR vesicles isolated from sheep hearts, Wei *et al*. discovered that CASQ2 increases the open probability of both RyR1 and RyR2 channels [42]. The interaction between CASQ2 and RyR2 may contribute to the refractory period of Ca^{2+} release [43]. The mutation of CASQ2 might affect the Ca^{2+}-binding ability, causing intra-SR Ca^{2+} concentration to rise much faster than normal. Thus, this loss of Ca^{2+} buffering may easily trigger RyR2 to open and cause CPVT. Ng *et al*. discovered numerous CASQ2 mutations, such as CASQ2 R251H, CASQ2 K180R, CASQ2 W361R, CASQ2 R33*Q, CASQ2 E39*, CASQ2 D340*, CASQ2 S173I, and CASQ2 D325E. Using turbidity assays, they discovered that CASQ2 D325E and CASQ2 K180R have filament defects with half capability of Ca^{2+} binding. CASQ2 R33Q and CASQ2 Y55C completely lose their ability to bind with Ca^{2+}. CASQ2 P308L and CASQ2 S173I have a similar situation to CASQ2 Y55C, but they restore around 25% of its capability 20 min after Ca^{2+} addition. CASQ2 R251H seems to have no effect on Ca^{2+} binding in normal pH, while losing about 25% of capability in pH 5.6, which is closer to physiological condition near the SR luminal membrane [44]. Postma *et al.* reported that three mutations in *CASQ2* in three CPVT families, a nonsense R33X, a splicing 532 + 1 G > A, and a 1-bp deletion, 62delA, are thought to induce premature stop codons. Three mutations share similar phenotypic variations. In total 16 heterozygous carriers of these mutations, only two of them have VAs on EKG during exercise tests and the rest of them are devoid of clinical symptoms or EKG anomalies [45].

Catecholaminergic polymorphic ventricular tachycardia-associated calmodulin mutation

CaM, an essential Ca^{2+} sensing, is a signal-transducing protein commonly conserved in eukaryotes and consists of four classical Ca^{2+}-binding EF-hands (EF1–4) located in two globular N-terminal (N-lobe) and C-terminal (C-lobe) domains connected by a short flexible linker region. These separated EF-hands can bind to Ca^{2+} with very high cooperativity that induces a structural modification, forming a Ca^{2+}/CaM complex and severing its universal function: amplification of the Ca^{2+} signal [22,46]. In the heart, CaM can modulate the gating of RyR2, L-type Ca^{2+}, Na^{+}, and K^{+} channels and trigger CaMKII [20,23,47]. It has been known that CaM plays a critical role in E-C coupling, and mutation in this protein is highly associated with CPVT and LQTs. CaM is encoded by three different genes, *CALM1*, *CALM2*, and *CALM3*. Mutation in these genes is called calmodulinopathies, and most of them are associated with life-threatening cardiac disorders, such as LQTs and CPVT [46]. There are many variations in CaM that have been associated with CPVT or CPVT/LQTs mix phenotypes, including CaM N53I, CaM N97I, CaM N98S, CaM F89L, CaM D95V, CaM D130G, CaM D132E, CaM E141G, and CaM Q136P [48-51]. Holt *et al*. found that structures of the arrhythmogenic CaM N53I variants are highly similar to wild-type the CaM, and variation in N53I alone can alter the intramolecular dynamics of CaM N-domain. Thus, it changes the interaction between the CaM N-domain and RyR2, thereby causing arrhythmia and CPVT [48]. Analyzing the crystal structure of various genetic modified CaM associated with LQTs, CPVT, or LQTs/CPVT mix from purified IQ domain of human $Ca_{V1.2}$ and full length human CaM, Wang *et al*. discussed the interaction changes between CaM and $Ca_V1.2$ IQ domain. CaM D130G completely separates EF-hands within the C-lobe and loses Ca^{2+} binding in EF-hand 4 [46]. CaM Q136P severely reduces the affinity for the IQ domain. CaM N98S creates different conformations with either one or two Ca^{2+} bound to the C-lobe that possibly disrupts the cooperativity [46,49]. CaM N54I is lobe variant which has no major changes in complexity with the IQ domain but causes severe stress-induced arrhythmia [46,49]. Using heterozygous CaM N98S transgenic mice generated by using CRISPR/Cas9 technology, Tsai *et al*. reported that this variation exhibits sinus bradycardia, QTc interval prolongation, and catecholaminergic bidirectional VT. Furthermore, CaM N98S significantly increases the peak density and induces slow inactivation and left shift of the activation curve of $I_{Ca.L}$ during β-adrenergic stimulation. They proposed that tachycardia-induced DAD and pause-dependent EAD originated in the His–Purkinje network and ventricular

myocytes via reentry or focal mechanisms constitute potential sources of arrhythmia in CaM N98S heart [52].

Catecholaminergic polymorphic ventricular tachycardia-associated calcium/calmodulin-dependent protein kinase II mutation

CaMKII, a multifunctional serine/threonine kinase, mediates multiple physiological responses [53]. There are four different isoforms of CaMKII (α, β, γ, and δ) with different Ca^{2+}/CaM binding affinity (γ > β > δ > α). CaMKIIδ and CaMKIIγ are predominantly expressed in the heart [54]. It can not only regulate numerous ion channels directly but also phosphorylate PLB and activate SERCA [55,56]. Endogenous CaMKII can phosphorylate RyR2, hence increasing its sensitivity to Ca^{2+}-dependent activation and the frequency of Ca^{2+} sparks [34,56]. This action leads to RyR2-mediated SR Ca^{2+} leak, subsequently causing DAD, and thereby triggers severe cardiac arrhythmias and CPVT [57]. The activation of CaMKII is strictly regulated by two sets of highly conserved phosphorylation sites in the regulatory segment (Thr 286 and Thr 305/Thr 306) that are blocked by the regulatory segment and released once Ca^{2+}/CaM attaches to CaMKII. CaMKII mutation in its own structure rarely occurs in CPVT or other inherited arrhythmias. Several studies suggested that using viral vector to deliver gene fragments, which encode CaMKII inhibitor peptides, can be a potential therapeutic strategy for inherited arrhythmias. Bezzerides *et al*. used an adeno-associated viral vector fused with CaMKII inhibitory peptide (autocamptide-2 related inhibitor peptide [AIP]) and systemically delivered to heterozygous RyR2 R176Q mice. The CaMKII inhibitory peptide is designed to selectively inhibit cardiac CaMKII due to the modified gene, which can only be expressed from a cardiomyocyte selective promoter. They reported that the inhibition of CaMKII successfully suppressed VA induced by either β-adrenergic stimulation or programmed ventricular pacing, without significantly proarrhythmic effect. They also reported that CaMKII inhibition reverses the arrhythmia phenotype in human CPVT iPSC-CM models with different pathogenic mutations [58]. These findings suggested that upon various genetic mutations in CPVT, administration of CaMKII inhibitor peptide (AIP) delivered by viral vector might be an effective therapeutic approach.

Catecholaminergic polymorphic ventricular tachycardia-associated triadin mutations

Triadin, which is expressed in the striated muscle, has at least three isoforms (Trisk 95, Trisk 51, and Trisk 32) due to the alternative splicing of the *TRDN* gene [59]. Trisk 32 is majorly expressed in cardiac muscle. CASQ2 is anchored to RyR2 by triadin and junction, forming a quaternary complex within SR [41,43,60]. It has been shown that CPVT patients with TRDN mutations all experienced cardiac arrest at a very young age (<6 years), suggesting a severe CPVT phenotype due to triadin mutations [59,61-63]. This mutation identified in CPVT patients is homozygous or compound heterozygous [59,61,62,64]. Using COS-7 cells and cardiomyocytes of triadin-knockout mouse, Roux-Buisson *et al*. reported that the mutation of Trisk 32 produces the mutant protein Trisk 32 T59R. They reported that Trisk 32 T59R is very unstable and tends to be degraded, suggesting that mutation of Trisk 32 T59R results in the absence of triadin and the cause of CPVT. They also discovered two other variations of the triadin in two CPVT families: p.D18Afs*13 and p.Q205*. These mutations also lead to the absence of triadin [61]. However, using triadin knockout mice and adeno-associated virus, Cacheux *et al*. demonstrated that Trisk 32 T59R does not abolish the ability of triadin to interact with CSQ2 as a chaperone. They also reported that loss of triadin is associated with an 80% reduction of CSQ2, a 30% reduction in RyR2, and a reduction in the SR Ca^{2+} load [63]. Using genome sequencing and a TRDN gene-specific trio analysis on RNA and protein isolated from patient-specific human iPSC-CMs, Clemens *et al*. discovered that alternative splicing on exon 6a-containing TRDN transcript in the normal heart leads to the absence of triadin, thereby causing triadin-knockout syndrome [65].

Catecholaminergic polymorphic ventricular tachycardia-associated HRC mutations

Similar to CASQ2, histidine-rich Ca^{2+} binding protein (HRC) also has low affinity and high capacity of Ca^{2+} binding and is expressed predominantly in the cardiac SR lumen of the striated muscle [66]. The interaction between HRC and triadin is Ca^{2+} sensitive [66,67]. Using cardiac homogenates from wild-type mice and postmortem human specimens, Arvanitis *et al*. discovered that the binding affinity of HRC to SERCA and triadin is very distinct. A recent study reported that the affinity of HRC for triadin increases as Ca^{2+} concentration rises from 10^{-8} to 10^{-3} M. The peak affinity between HRC and SERCA appears at pKa 7, whereas between HRC and triadin appears at pKa 4. They also reported that an increase of Ca^{2+} concentration in SR reduces the interaction between HRC and SERCA, whereas the interaction between HRC and triadin is increased [66]. These findings suggested that HRC may sense and regulate Ca^{2+} uptake and release, hence constantly changing the binding partners in SR during E-C coupling [66,68]. Using HRC and CASQ2 double-knockout mice, Liu *et al*. reported that the absence of HRC and CASQ2 can ameliorate the predisposition to VA and arrhythmogenic Ca^{2+} waves. They proposed that HRC enhances the RyR2 activity through facilitating RyR2 recovery from refractoriness and CASQ2 stabilizes RyR2 rendering it refractory in the diastolic phase. Thus, the losses of Ca^{2+} buffering of CASQ2 combined with normal HRC can lead to RyR2 frequently open and cause CPVT. Surprisingly, with HRC also absent in this scenario, it ablates CPVT [60]. Their discovery suggested a new thinking pathway for CPVT patients with CASQ2 mutation. Using HEK-293 cells, Zhang *et al*. demonstrated that HRC S96A leads to an increase in spontaneous Ca^{2+} release and ultimately cause arrhythmias by disrupting the regulation of intra-store free Ca^{2+}. They proposed that this phenomenon is primarily due to an impaired ability to act as an effective bulk and local micro-domain store Ca^{2+} buffer [69].

Catecholaminergic polymorphic ventricular tachycardia-associated TECRL mutations

TECRL is a 12 exon gene located on chromosomal segment 4q13. The protein encoded by this gene is called trans-2,3-enoyl-CoA reductase-like, which belongs to the steroid 5-alpha reductase and is predominantly expressed in the ER of cardiomyocytes [27].

The trans-2,3-enoyl-CoA reductase-like plays a crucial role in intracellular Ca^{2+} regulation. *TECRL* mutation presents a mixed phenotype of CPVT and LQTs termed CPVT 3, and variants in *TECRL* may cause up to 5% of all CPVT cases [3,27]. Using DNA samples from CPVT patients, Moscu-Gregor *et al*. discovered that a point mutation in Gln139* results in a premature stop codon and loss of the trans-2,3-enoyl-CoA reductase-like function. Homozygous variants NM_001010874.4:c. 869C>A, p.Pro290His from CPVT patients are probably leading to an altered folding of the 3-oxo-5-alpha steroid 4-dehydrogenase domain of the trans-2,3-enoyl-CoA reductase-like. However, the large population cohort study of this variation has not been done yet. It only has been predicted by silico-prediction as pathogenic [27]. NM_001010874.4:c. 893T>C, p.(Val298Ala), and c. 926C>A, p.(Ser309*) are suggested to affect the transmembrane complex and parts of the 3-oxo-5-alpha steroid 4-dehydrogenase domain and have a high possibility of a loss of function [27]. Devalla *et al*. reported that two French–Canadian probands carried identical homozygous variants (p.Arg196Gln), which were diagnosed with CPVT. They compared the difference between homozygous variation and healthy persons and reported that the intracellular Ca^{2+} transient is slower than normal in the rising and decay phase. Moreover, the SR Ca^{2+} storage is lower than normal. The homozygous variation presents with longer AP and significantly increases the propensity for triggered activity by noradrenaline [70].

Management of catecholaminergic polymorphic ventricular tachycardia

Current therapeutic management for patients with CPVT includes beta-blockers, flecainide, verapamil, ivabradine, left cardiac sympathetic denervation (LCSD), bilateral thoracoscopic sympathectomy via a minimally invasive video-assisted thoracoscopic surgery (VATS-LCSD), implantable cardiac defibrillators, and catheter ablation [3,71]. Above all, limiting physical exercise, reducing or preventing stress, or emotion-induced matters should be the top priority [72].

According to Heart Rhythm Society/European Heart Rhythm Association/Asia Pacific Heart Rhythm Society (HRS/EHRA/APHRS) recommendation, beta-blockers without intrinsic sympathomimetic activity, such as nadolol, propranolol, and carvedilol, are the first-line therapeutic options for CPVT patients [3,71]. For prophylactic management, nadolol is more preferable than other beta-blockers due to its long-acting characteristic [3,71]. For countries that nadolol is not available, a nonselective beta-blocker, such as propranolol, is equally effective as nadolol [71]. Flecainide is capable of reducing the frequency of spontaneous Ca^{2+} waves and RyR2-mediated Ca^{2+} waves *in vitro*. Moreover, flecainide can inhibit I_{Na} and dramatically reduce the number of ectopic beats during isoproterenol-induced CPVT [73]. Flecainide has been reported to successfully reduce exercise-induced VAs in a 33-CPVT patient trial, and HRS/EHRA/APHRS has recommended it as the first addition to beta-blockers when control of arrhythmias is incomplete [3,74].

Ivabradine, a funny channel pacemaker current inhibitor target specifically on the pacemaker activity of the sinoatrial node, has been recommended for heart failure treatment. In 2020, Kohli *et al*. reported that an 18-year-old male with a large in-frame RYR2 E3D, malignant syncope, and CPVT was given nadolol and flecainide and underwent sympathectomy. However, these managements remain ineffective. His VA was successfully suppressed after the initiation of ivabradine treatment. Kohli *et al*. suggested that ivabradine could be an important add-on therapy for CPVT patients, who are drug-refractory or unable to continue conventional therapies [75]. Using CASQ2 D307H/D307H mice and iPSC-CM, Bueno-Levy *et al*. discovered TRAM-34, a Ca^{2+}-activated potassium channel selective blocker, can manage the depolarization of maximal diastolic potential, reduce the heart rate, and attenuate VAs in CPVT patients [76]. All three drugs still need further investigation and human trials to understand the exact mechanism, potential side effects, and adaptability on CPVT patients. Verapamil is also a beta-blocking agent that has been recommended for women during pregnancy and postpartum with LQTs or CPVT [74]. Verapamil has been shown to be beneficial for some CPVT patients in short-term follow-up, whereas its long-term effect remains controversial [71,77]. Cather ablation is commonly used in various VAs [77]. It triggers VF in patients with refractory CPVT and could be used as adjunctive therapy for bidirectional ventricular premature beats [71]. However, it is not recommended for standard CPVT treatment due to the vast experimental trials still not available [71]. LCSD is another surgical management for CPVT and has been shown to be beneficial in short-term arrhythmic events recovery [71]. VAT-LCSD, an improved version of LCSD, can operate via minimal invasion, resulting in significantly less morbidity and a shorted hospital stay. It is suitable for patients in recurrent VTs with congenital LQTs and CPVT [3]. Furthermore, it has been shown to have an anti-arrhythmic and anti-fibrillatory effect and thereby provides a critical adjunct to existing medical therapies and should be considered for all patients with life-threatening refractory arrhythmias, especially those patients on maximal medical therapy [3]. However, both LCSD and VAT-LCSD are lacking massive clinical trials and long-term follow-up results to support its capability of improving quality of life, effectiveness, and safety in CPVT patients [3,71].

Conclusion

In this review, we provide a brief introduction of the molecular mechanism associated with CPVT as presented in Figure 1. We also provide a summary of those CPVT-related protein mutations

Table 1: Summary of catecholaminergic polymorphic ventricular tachycardia-related protein's mutation and their biophysical effects in catecholaminergic polymorphic ventricular tachycardia

Protein	Gene	Mutation	Pathology	Reference
RyR2	RYR2	c. 12343C>T (p.L4115F)	Shorter AP duration, increase DAD, increase EAD, tachyarrhythmia	[35]
		c. 168-301_ c. 273+722del1128 (E3D)	Shorter AP duration, lower AP amplitude, lower upstroke velocity, increase depolarized diastolic potential, increase EAD, increase DAD, tachyarrthymia	[35]
		c. 11836G>A (p.Gly3946Ser)	CPVT	[9]
		c. 6982C>T (p.Pro2328Ser)	>10-fold shift in the AC50 for Ca^{2+}-activation (from~3.5 μM Ca^{2+}in WT RyR2 to~320 nM in P2328S channels), >1000-fold shift in the IC50 for inactivation (from~50 mM in WT channels to≤7 μM in P2328S channels)	[36]
		c. 527G>T (p.Arg176Leu)	Increase DAD	[39]
		c. 13489C>T (p.Arg4496Cys)	More frequent (>2X) and higher amplitude (2X~3X) of spontaneous Ca^{2+}release events	[37]
CASQ2	*CASQ2*	c. 752G>A (p.Arg251His)	Filamentation defects (only in pH 5.6, partial malfunction)	[44]
		c. 539A>G (p.Lys180Arg)	CPVT	
		c. 1081T>A (p.Trp361Arg)	Filamentation defects (total malfunction)	[44]
		c. 98G>A (p.Arg33Gln)	Filamentation defects (total malfunction)	[44]
		c. 115G>T (p.Glu39Ter)	CPVT	
		c. 1017dup (p.Asp340Ter)	CPVT	
		c. 518G>T (p.Ser173Ile)	Filamentation defects (partial malfunction)	[44]
		(p.Asp325Glu)	Filamentation defects (partial malfunction)	[44]
		c. 164A>G (p.Tyr55Cys)	Filamentation defects (partial malfunction)	[44]
		c. 923C>T (p.Pro308Leu)	Filamentation defects (partial malfunction)	[44]
		c. 97C>T (p.Arg33Ter)	CPVT	
Calmodulin	*CALM1*	N54I, N53I	Significantly destabilize the CaM N-domain	[48,78,79]
		F90L, F89L	Decrease CaMKII activity	[80]
		N98S, N97S	Decrease CaMKII activity, partial CDI	[78,79,81]
		D130G, D129G	Complete loss of Ca^{2+}binding in EF-hand 4, diminished CDI, can't activate CaMKII	[46,79,81-83]
		E141G, E140G	Dominant loss of CDI	[84]
	CALM2	N98I, N98I	CPVT, LQTs	[85]
		N98S, N97S	Decrease CaMKII activity, partial CDI	[79,81,85-87]
		D96V, D95V	Loss of CDI, decrease CaMKII activity	[82]
		D132E, D131E	CPVT, LQTs	[85]
		Q136P, Q135P	Severely reduce affinity for the IQ domain	[46]
		D130G, D129G	Diminished CDI, can't activate CaMKII	[79,81,83,84]
	CALM3	D130G, D129G	Completely loss of Ca^{2+}binding in EF-hand 4	[79,81,83,88,89]
Triadin	*TRDN*	c. 53_56delACAG (p.Asp18AlafsTer13)	Likely absence	[60,65]
		c. 613C-T (p.Q205X)	Likely absence	[60,65]
		c. 176C-G (p.T59R)	Instable for degradation, likely absence	[60,65]
trans-2,3-enoyl-CoA reductase-like	*TECRL*	c. 415C>T, (p.Gln139*)	Likely pathogenic	[27]
		c. 893T>C, (p.Val298Ala)	Predicted to be pathogenic	[27]
		c. 869C>A, (p.Pro290His)	Predicted to be pathogenic	[27]
		c. 587G>A (p.Arg196Gln)	Increase AP, lower SR Ca^{2+}storage	[70]
		c. 926C>A, (p.Ser309*)	Likely loss of function	[27]

CDI: Ca^{2+}-dependent inactivation, DAD: Delayed after depolarization, CPVT: Catecholaminergic polymorphic ventricular tachycardia, AP: Action potential, RyR2: Ryanodine receptors 2, EAD: Early after depolarization, LQTs: Long QT syndrome, WT: Wild type, *: deletion

and their influence in CPVT as shown in Table 1. In conclusion, CPVT is an early onset disease that usually presents with episodic syncope, arrhythmia, or even sudden cardiac death. Early diagnosis with exercise stress tests and genetic tests are beneficial for patients and their families. Various Ca^{2+} handling protein mutations cause severe Ca^{2+} imbalance within cardiomyocytes. The exact mechanism underlying mutation protein-induced disturbances of Ca^{2+} handling is still not fully understood. The current treatments of CPVT have been focused on symptom treatments. As the knowledge of CPVT has grown massively, the experimental gene therapy may provide us a new therapeutic strategy.

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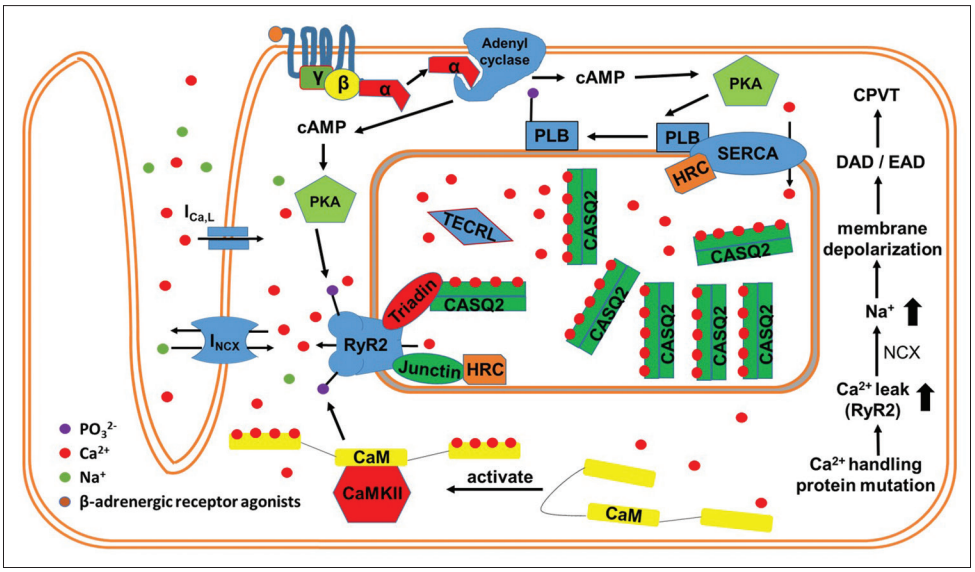

Figure 1: The molecular mechanism of Ca^{2+} handling related to catecholaminergic polymorphic ventricular tachycardia

Conflicts of interest

There are no conflicts of interest.

References

1. Priori SG, Napolitano C, Memmi M, Colombi B, Drago F, Gasparini M, et al. Clinical and molecular characterization of patients with catecholaminergic polymorphic ventricular tachycardia. Circulation 2002;106:69-74.
2. Schwartz PJ, Crotti L, Insolia R. Long-QT syndrome: From genetics to management. Circ Arrhythm Electrophysiol 2012;5:868-77.
3. Pérez-Riera AR, Barbosa-Barros R, de Rezende Barbosa MP, Daminello-Raimundo R, de Lucca AA Jr., de Abreu LC. Catecholaminergic polymorphic ventricular tachycardia, an update. Ann Noninvasive Electrocardiol 2018;23:e12512.
4. Jalloul Y, Refaat MM. Novel variants in TECRL cause catecholaminergic polymorphic ventricular tachycardia. J Cardiovasc Electrophysiol 2020;31:1536-8.
5. Allouis M, Probst V, Jaafar P, Schott JJ, Le Marec H. Unusual clinical presentation in a family with catecholaminergic polymorphic ventricular tachycardia due to a G14876A ryanodine receptor gene mutation. Am J Cardiol 2005;95:700-2.
6. Celiker A, Erdoğan I, Karagöz T, Ozer S. Clinical experiences of patients with catecholaminergic polymorphic ventricular tachycardia. Cardiol Young 2009;19:45-52.
7. Marjamaa A, Hiippala A, Arrhenius B, Lahtinen AM, Kontula K, Toivonen L, et al. Intravenous epinephrine infusion test in diagnosis of catecholaminergic polymorphic ventricular tachycardia. J Cardiovasc Electrophysiol 2012;23:194-9.
8. Marjamaa A, Laitinen-Forsblom P, Lahtinen AM, Viitasalo M, Toivonen L, Kontula K, et al. Search for cardiac calcium cycling gene mutations in familial ventricular arrhythmias resembling catecholaminergic polymorphic ventricular tachycardia. BMC Med Genet 2009;10:12.
9. Gallegos-Cortez A, Alonso-Ortiz N, Antunez-Argüellez E, Villarreal-Molina T, Totomoch-Serra A, Iturralde-Torres P, et al. Catecholaminergic polymorphic ventricular tachycardia due to *de novo* RyR2 mutation: Recreational cycling as a trigger of lethal arrhythmias. Arch Med Sci 2020;16:466-70.
10. Singh M, Morin DP, Link MS. Sudden cardiac death in long QT syndrome (LQTS), Brugada syndrome, and catecholaminergic polymorphic ventricular tachycardia (CPVT). Prog Cardiovasc Dis 2019;62:227-34.
11. Miyata K, Ohno S, Itoh H, Horie M. Bradycardia is a specific phenotype of catecholaminergic polymorphic ventricular tachycardia induced by RYR2 mutations. Intern Med 2018;57:1813-7.
12. Members AT, Priori SG, Blomström-Lundqvist C, Mazzanti A, Blom N, Borggrefe M, 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). Europace 2015;17:1601-87.
13. Hayashi M, Denjoy I, Extramiana F, Maltret A, Buisson NR, Lupoglazoff JM, et al. Incidence and risk factors of arrhythmic events in catecholaminergic polymorphic ventricular tachycardia. Circulation 2009;119:2426-34.
14. Letsas KP, Prappa E, Bazoukis G, Lioni L, Pantou MP, Gourzi P, et al. A novel variant of RyR2 gene in a family misdiagnosed as congenital long QT syndrome: The importance of genetic testing. J Electrocardiol 2020;60:8-11.
15. Campuzano O, Sarquella-Brugada G, Arbelo E, Cesar S, Jordà P, Pérez-Serra A, et al. Genetic variants as sudden-death risk markers in inherited arrhythmogenic syndromes: Personalized genetic interpretation. J Clin Med 2020;9:1866.
16. Kukla P, Biernacka EK, Baranchuk A, Jastrzebski M, Jagodzinska M. Electrocardiogram in Andersen-Tawil syndrome. New electrocardiographic criteria for diagnosis of type-1 Andersen-Tawil syndrome. Curr Cardiol Rev 2014;10:222-8.
17. Priori SG, Chen SR. Inherited dysfunction of sarcoplasmic reticulum Ca^{2+}handling and arrhythmogenesis. Circ Res 2011;108:871-83.
18. Jiang D, Wang R, Xiao B, Kong H, Hunt DJ, Choi P, et al. Enhanced store overload-induced Ca^{2+}release and channel sensitivity to luminal Ca^{2+}activation are common defects of RyR2 mutations linked to ventricular tachycardia and sudden death. Circ Res 2005;97:1173-81.
19. Jiang D, Xiao B, Yang D, Wang R, Choi P, Zhang L, et al. RyR2 mutations linked to ventricular tachycardia and sudden death reduce the threshold for store-overload-induced Ca^{2+}release (SOICR). Proc Natl Acad Sci U S A 2004;101:13062-7.
20. Denniss AL, Dashwood AM, Molenaar P, Beard NA. Sarcoplasmic reticulum calcium mishandling: Central tenet in heart failure? Biophys

Rev 2020;12:865-78.

21. Refaat MM, Aouizerat BE, Pullinger CR, Malloy M, Kane J, Tseng ZH. Association of CASQ2 polymorphisms with sudden cardiac arrest and heart failure in patients with coronary artery disease. Heart Rhythm 2014;11:646-52.
22. Glukhov AV, Kalyanasundaram A, Lou Q, Hage LT, Hansen BJ, Belevych AE, et al. Calsequestrin 2 deletion causes sinoatrial node dysfunction and atrial arrhythmias associated with altered sarcoplasmic reticulum calcium cycling and degenerative fibrosis within the mouse atrial pacemaker complex1. Eur Heart J 2015;36:686-97.
23. Faggioni M, Savio-Galimberti E, Venkataraman R, Hwang HS, Kannankeril PJ, Darbar D, et al. Suppression of spontaneous ca elevations prevents atrial fibrillation in calsequestrin 2-null hearts. Circ Arrhythm Electrophysiol 2014;7:313-20.
24. Refaat MM, Hotait M, London B. Genetics of sudden cardiac death. Curr Cardiol Rep 2015;17:606.
25. Márquez MF, Totomoch-Serra A, Rueda A, Avelino-Cruz JE, Gallegos-Cortez A. Basic and clinical insights in catecholaminergic (familial) polymorphic ventricular tachycardia. Rev Invest Clin 2019;71:226-36.
26. Shigemizu D, Aiba T, Nakagawa H, Ozaki K, Miya F, Satake W, et al. Exome analyses of long QT syndrome reveal candidate pathogenic mutations in calmodulin-interacting genes. PLoS One 2015;10:e0130329.
27. Moscu-Gregor A, Marschall C, Müntjes C, Schönecker A, Schuessler-Hahn F, Hohendanner F, et al. Novel variants in TECRL cause recessive inherited CPVT type 3 with severe and variable clinical symptoms. J Cardiovasc Electrophysiol 2020;31:1527-35.
28. Prestle J, Janssen PM, Janssen AP, Zeitz O, Lehnart SE, Bruce L, et al. Overexpression of FK506-binding protein FKBP12.6 in cardiomyocytes reduces ryanodine receptor-mediated Ca(2+) leak from the sarcoplasmic reticulum and increases contractility. Circ Res 2001;88:188-94.
29. Gonano LA, Vila Petroff M. Direct modulation of RyR2 leading to a TRICky Ca^{2+} balance: The effects of TRIC-A on cardiac muscle. Circ Res 2020;126:436-8.
30. Xiao B, Zhong G, Obayashi M, Yang D, Chen K, Walsh MP, et al. Ser-2030, but not Ser-2808, is the major phosphorylation site in cardiac ryanodine receptors responding to protein kinase A activation upon β-adrenergic stimulation in normal and failing hearts. Biochem J 2006;396:7-16.
31. Dashwood A, Cheesman E, Beard N, Haqqani H, Wong YW, Molenaar P. Understanding how phosphorylation and redox modifications regulate cardiac ryanodine receptor type 2 activity to produce an arrhythmogenic phenotype in advanced heart failure. ACS Pharmacol Transl Sci 2020;3:563-82.
32. Marx SO, Reiken S, Hisamatsu Y, Jayaraman T, Burkhoff D, Rosemblit N, et al. PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): Defective regulation in failing hearts. Cell 2000;101:365-76.
33. Carter S, Colyer J, Sitsapesan R. Maximum phosphorylation of the cardiac ryanodine receptor at serine-2809 by protein kinase a produces unique modifications to channel gating and conductance not observed at lower levels of phosphorylation. Circ Res 2006;98:1506-13.
34. Wehrens XH, Lehnart SE, Reiken SR, Marks AR. Ca^{2+}/calmodulin-dependent protein kinase II phosphorylation regulates the cardiac ryanodine receptor. Circ Res 2004;94:e61-70.
35. Pölönen RP, Swan H, Aalto-Setälä K. Mutation-specific differences in arrhythmias and drug responses in CPVT patients: Simultaneous patch clamp and video imaging of iPSC derived cardiomyocytes. Mol Biol Rep 2020;47:1067-77.
36. Salvage SC, Gallant EM, Beard NA, Ahmad S, Valli H, Fraser JA, et al. Ion channel gating in cardiac ryanodine receptors from the arrhythmic RyR2-P2328S mouse. J Cell Sci 2019;132:jcs229039.
37. 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.
38. 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.
39. Tung M, Van Petegem F, Lauson S, Collier A, Hodgkinson K, Fernandez B, et al. Cardiac arrest in a mother and daughter and the identification of a novel RYR2 variant, predisposing to low penetrant catecholaminergic polymorphic ventricular tachycardia in a four-generation Canadian family. Mol Genet Genomic Med 2020;8:e1151.
40. Wleklinski MJ, Kannankeril PJ, Knollmann BC. Molecular and tissue mechanisms of catecholaminergic polymorphic ventricular tachycardia. J Physiol 2020;598:2817-34.
41. Katz G, Arad M, Eldar M. Catecholaminergic polymorphic ventricular tachycardia from bedside to bench and beyond. Curr Probl Cardiol 2009;34:9-43.
42. Wei L, Hanna AD, Beard NA, Dulhunty AF. Unique isoform-specific properties of calsequestrin in the heart and skeletal muscle. Cell Calcium 2009;45:474-84.
43. Györke S, Stevens SC, Terentyev D. Cardiac calsequestrin: Quest inside the SR. J Physiol 2009;587:3091-4.
44. Ng K, Titus EW, Lieve KV, Roston TM, Mazzanti A, Deiter FH, et al. An international multi-center evaluation of inheritance patterns, arrhythmic risks, and underlying mechanisms of CASQ2-catecholaminergic polymorphic ventricular tachycardia. Circulation 2020;142:932-47.
45. Postma AV, Denjoy I, Hoorntje TM, Lupoglazoff JM, Da Costa A, Sebillon P, et al. Absence of calsequestrin 2 causes severe forms of catecholaminergic polymorphic ventricular tachycardia. Circ Res 2002;91:e21-6.
46. Wang K, Brohus M, Holt C, Overgaard MT, Wimmer R, Van Petegem F. Arrhythmia mutations in calmodulin can disrupt cooperativity of Ca^{2+}binding and cause misfolding. J Physiol 2020;598:1169-86.
47. Uchinoumi H, Yang Y, Oda T, Li N, Alsina KM, Puglisi JL, et al. CaMKII-dependent phosphorylation of RyR2 promotes targetable pathological RyR2 conformational shift. J Mol Cell Cardiol 2016;98:62-72.
48. Holt C, Hamborg L, Lau K, Brohus M, Sørensen AB, Larsen KT, et al. The arrhythmogenic N53I variant subtly changes the structure and dynamics in the calmodulin N-terminal domain, altering its interaction with the cardiac ryanodine receptor. J Biol Chem 2020;295:7620-34.
49. Wang K, Brohus M, Holt C, Overgaard MT, Wimmer R, Van Petegem F. Arrhythmogenic calmodulin mutations can disrupt the globular structure and uncouple Ca^{2+}binding cooperativity. Biophys J 2020;118:106a.
50. Su J, Gao Q, Yu L, Sun X, Feng R, Shao D, et al. The LQT-associated calmodulin mutant E141G induces disturbed Ca^{2+}-dependent binding and a flickering gating mode of the $Ca_V1.2$ channel. Am J Physiol Cell Physiol 2020;318:C991-1004.
51. Chazin WJ, Johnson CN. Calmodulin mutations associated with heart arrhythmia: A status report. Int J Mol Sci 2020;21:1418.
52. 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.
53. Grimm M, Brown JH. β-Adrenergic receptor signaling in the heart: Role of CaMKII. J Mol Cell Cardiol 2010;48:322-30.
54. Mustroph J, Drzymalski M, Baier M, Pabel S, Biedermann A, Memmel B, et al. The oral Ca/calmodulin-dependent kinase II inhibitor RA608 improves contractile function and prevents arrhythmias in heart failure. ESC Heart Fail 2020;7:2871-83.
55. Greer-Short A, Musa H, Alsina KM, Ni L, Word TA, Reynolds JO, et al. Calmodulin kinase II regulates atrial myocyte late sodium current, calcium handling, and atrial arrhythmia. Heart Rhythm 2020;17:503-11.

56. Guo T, Zhang T, Mestril R, Bers DM. Ca^{2+}/Calmodulin-dependent protein kinase II phosphorylation of ryanodine receptor does affect calcium sparks in mouse ventricular myocytes. Circ Res 2006;99:398-406.
57. Voigt N, Li N, Wang Q, Wang W, Trafford AW, Abu-Taha I, et al. Enhanced sarcoplasmic reticulum Ca^{2+}leak and increased Na+-Ca^{2+}exchanger function underlie delayed afterdepolarizations in patients with chronic atrial fibrillation. Circulation 2012;125:2059-70.
58. Bezzerides VJ, Caballero A, Wang S, Ai Y, Hylind RJ, Lu F, et al. Gene therapy for catecholaminergic polymorphic ventricular tachycardia by inhibition of Ca^{2+}/calmodulin-dependent kinase II. Circulation 2019;140:405-19.
59. Rooryck C, Kyndt F, Bozon D, Roux-Buisson N, Sacher F, Probst V, et al. New family with catecholaminergic polymorphic ventricular tachycardia linked to the triadin gene. J Cardiovasc Electrophysiol 2015;26:1146-50.
60. Liu B, Ho HT, Brunello L, Unudurthi SD, Lou Q, Belevych AE, et al. Ablation of HRC alleviates cardiac arrhythmia and improves abnormal Ca handling in CASQ2 knockout mice prone to CPVT. Cardiovasc Res 2015;108:299-311.
61. Roux-Buisson N, Cacheux M, Fourest-Lieuvin A, Fauconnier J, Brocard J, Denjoy I, 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;21:2759-67.
62. Walsh MA, Stuart AG, Schlecht HB, James AF, Hancox JC, Newbury-Ecob RA. Compound heterozygous triadin mutation causing cardiac arrest in two siblings. Pacing Clin Electrophysiol 2016;39:497-501.
63. Cacheux M, Fauconnier J, Thireau J, Osseni A, Brocard J, Roux-Buisson N, et al. Interplay between triadin and calsequestrin in the pathogenesis of CPVT in the mouse. Mol Ther 2020;28:171-9.
64. Marty I. Triadin regulation of the ryanodine receptor complex. J Physiol 2015;593:3261-6.
65. Clemens DJ, Tester DJ, Marty I, Ackerman MJ. Phenotype-guided whole genome analysis in a patient with genetically elusive long QT syndrome yields a novel TRDN-encoded triadin pathogenetic substrate for triadin knockout syndrome and reveals a novel primate-specific cardiac TRDN transcript. Heart Rhythm 2020;17:1017-24.
66. Arvanitis DA, Vafiadaki E, Fan GC, Mitton BA, Gregory KN, Del Monte F, et al. Histidine-rich Ca-binding protein interacts with sarcoplasmic reticulum Ca-ATPase. Am J Physiol Heart Circ Physiol 2007;293:H1581-9.
67. Sacchetto R, Damiani E, Turcato F, Nori A, Margreth A. Ca^{2+}-dependent interaction of triadin with histidine-rich Ca^{2+}-binding protein carboxyl-terminal region. Biochem Biophys Res Commun 2001;289:1125-34.
68. Arvanitis DA, Vafiadaki E, Sanoudou D, Kranias EG. Histidine-rich calcium binding protein: The new regulator of sarcoplasmic reticulum calcium cycling. J Mol Cell Cardiol 2011;50:43-9.
69. Zhang JZ, McLay JC, Jones PP. The arrhythmogenic human HRC point mutation S96A leads to spontaneous Ca^{2+}release due to an impaired ability to buffer store Ca^{2+}. J Mol Cell Cardiol 2014;74:22-31.
70. Devalla HD, Gélinas R, Aburawi EH, Beqqali A, Goyette P, Freund C, et al. TECRL, a new life-threatening inherited arrhythmia gene associated with overlapping clinical features of both LQTS and CPVT. EMBO Mol Med 2016;8:1390-408.
71. 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.
72. Kawata H, Ohno S, Aiba T, Sakaguchi H, Miyazaki A, Sumitomo N, et al. Catecholaminergic polymorphic ventricular tachycardia (CPVT) associated with ryanodine receptor (RyR2) gene mutations- long-term prognosis after initiation of medical treatment. Circ J 2016;80:1907-15.
73. Kryshtal DO, Blackwell DJ, Smith AN, Batiste SM, Johnston JN, Knollmann BC. RyR2 inhibition by flecainide determines antiarrhythmic activity in CPVT. Biophys J 2020;118:567a.
74. van der Werf C, Kannankeril PJ, Sacher F, Krahn AD, Viskin S, Leenhardt A, et al. Flecainide therapy reduces exercise-induced ventricular arrhythmias in patients with catecholaminergic polymorphic ventricular tachycardia. J Am Coll Cardiol 2011;57:2244-54.
75. Kohli U, Aziz Z, Beaser AD, Nayak HM. Ventricular arrhythmia suppression with ivabradine in a patient with catecholaminergic polymorphic ventricular tachycardia refractory to nadolol, flecainide, and sympathectomy. Pacing Clin Electrophysiol 2020;43:527-33.
76. Bueno-Levy H, Weisbrod D, Yadin D, Haron-Khun S, Peretz A, Hochhauser E, et al. The hyperpolarization-activated cyclic-nucleotide-gated channel blocker ivabradine does not prevent arrhythmias in catecholaminergic polymorphic ventricular tachycardia. Front Pharmacol 2019;10:1566.
77. Rosso R, Kalman JM, Rogowski O, Diamant S, Birger A, Biner S, et al. Calcium channel blockers and beta-blockers versus beta-blockers alone for preventing exercise-induced arrhythmias in catecholaminergic polymorphic ventricular tachycardia. Heart Rhythm 2007;4:1149-54.
78. 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.
79. Berchtold MW, Zacharias T, Kulej K, Wang K, Torggler R, Jespersen T, et al. The arrhythmogenic calmodulin mutation D129G dysregulates cell growth, calmodulin-dependent kinase II activity, and cardiac function in zebrafish. J Biol Chem 2016;291:26636-46.
80. 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.
81. 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.
82. 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.
83. 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.
84. 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.
85. 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.
86. Jiménez-Jáimez J, Palomino Doza J, Ortega Á, Macías-Ruiz R, Perin F, Rodríguez-Vázquez del Rey MM, et al. Calmodulin 2 mutation N98S is associated with unexplained cardiac arrest in infants due to low clinical penetrance electrical disorders. PLoS One 2016;11:e0153851.
87. Fujita S, Nakagawa R, Futatani T, Igarashi N, Fuchigami T, Saito S, et al. Long QT syndrome with a *de novo* CALM2 mutation in a 4-year-old boy. Pediatr Int 2019;61:852-8.
88. Reed GJ, Boczek NJ, Etheridge SP, Ackerman MJ. CALM3 mutation associated with long QT syndrome. Heart Rhythm 2015;12:419-22.
89. 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.