Catecholaminergic Polymorphic Ventricular Tachycardia from Bedside to Bench and Beyond

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Abstract: Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a primary electrical myocardial disease characterized by exercise- and stress-related ventricular tachycardia manifested as syncope and sudden death. The disease has a heterogeneous genetic basis, with mutations in the cardiac Ryanodine Receptor channel (RyR2) gene accounting for an autosomal-dominant form (CPVT1) in approximately 50% and mutations in the cardiac calsequestrin gene (CASQ2) accounting for an autosomal-recessive form (CPVT2) in up to 2% of CPVT cases. Both RyR2 and calsequestrin are important participants in the cardiac cellular calcium homeostasis.

We review the physiology of the cardiac calcium homeostasis, including the cardiac excitation contraction coupling and myocyte calcium cycling. The pathophysiology of cardiac arrhythmias related to myocyte calcium handling and the effects of different modulators are discussed.

The putative derangements in myocyte calcium homeostasis responsible for CPVT, as well as the clinical manifestations and therapeutic options available, are described. (Curr Probl Cardiol 2009;34:9-43.)

Catecholaminergic polymorphic ventricular tachycardia (CPVT) belongs to a family of primary electrical heart diseases characterized by severe arrhythmias in young patients with apparently normal hearts.¹ The most prevalent and researched of this group of
diseases is the congenital long QT syndrome (LQTS), which also includes the Brugada syndrome, the short-coupled variant of torsade de pointes, the recently described short QT syndrome, and idiopathic ventricular fibrillation (VF).  

CPVT was first described in a case report in 1975. The late Philippe Coumel described four children with catecholamine-induced syncopal ventricular tachycardia (VT) in 1978. In 1995, a comprehensive study of CPVT by Leenhardt et al from the Coumel group described 21 children suffering from stress- or emotion-induced syncope (single or recurrent), with no evidence of structural heart disease and normal QT interval (see below). There was a family history of syncope or sudden death in seven patients, suggesting that CPVT has a genetic origin. However, no genetic analysis was reported.

Our understanding of the genetic basis of this disease began with a report by Swan et al who described two unrelated Finish families with a typical presentation of CPVT segregating in an autosomal-dominant mode. Symptoms in these families appeared at an average age of 21 years with a high mortality rate among affected individuals (31% by the age of 30 years). Using linkage analysis, they mapped the disease to chromosome 1q42-43. Priori et al and shortly afterwards Laitinen et al identified mutations in the cardiac ryanodine receptor gene (RyR2) in families suffering from this type of CPVT, now termed CPVT1. A recessive form of the disease has been described by Lahat et al from our group. We studied members of seven nuclear families belonging to a Bedouin tribe from the north of Israel with a history typical of CPVT. By means of genome-wide linkage analysis the disease was mapped to chromosome 1p13-21, and later, a missense mutation in a highly conserved region of the cardiac calsequestrin gene (CASQ2) was identified as the potential cause of this form of CPVT, now termed CPVT2.

Clinical Manifestations

As previously stated, the first comprehensive description of CPVT was provided in 1995 by Leenhardt et al, who described 21 patients (12 males), 20 of whom had suffered from syncopal episode(s). Almost half of the patients had been diagnosed as suffering from epilepsy prior to being correctly diagnosed with CPVT. Their age at first syncope ranged between 3 and 16 years (mean, 7.8 ± 4) and they had a slow resting heart rate of 60 ± 9 bpm. Physical examination, baseline electrocardiograms, and cardiac echocardiographic examinations were normal. Programmed electrical stimulation in six patients did not elicit significant arrhythmias. The hallmark of the disease was a reproducible form of polymorphic
ventricular tachycardia (PVT), which developed in a typical manner during exercise or emotion (Fig 1A and B). First, ventricular premature complexes (VPC) appeared at rates between 120 and 130 bpm (mean, 122 ± 13), sometimes with concurrent development of junctional rhythm. With continued catecholaminergic drive, the VPCs increased in number and PVT appeared. Fifteen patients developed salvos of bidirectional VT. In many patients a pattern of right bundle branch block with alternating left and right axis deviation was noted. On continued effort, very fast PVT followed, resulting in VF-like arrhythmia at the time of syncope.

**M. M. Scheinman and J. N. Weiss:** Other patients with genetic cardiac rhythm disorders may have exercise-induced provocation of serious ventricular arrhythmias. This is especially true for patients with the long QT syndrome (ie, LTQ1, LTQ4, and the Andersen–Tawil syndrome). The differentiation of these patients from those with CPVT relies on the facts that CPVT patients have normal QT (or QTc) intervals and because of the very stereotypic response to exercise as nicely summarized in the Leenhardt study.

Treatment with amiodarone and type I antiarrhythmic drugs was ineffective. Beta-receptor blockers (mostly nadolol) were very effective in preventing the PVTs. During a mean follow-up of 7 years, two patients died suddenly (during treatment with beta-receptor blockers) and two had
syncope (one when skipping or delaying a beta-receptor blocker drug, and the other with documented VF while treated with amiodarone). Seven patients (30%) had a family history of syncope or sudden death and autosomal-dominant heredity was suspected.

A subsequent significant contribution was made by Priori et al in 2002,11 who described 30 probands referred because of exercise- or emotion-related VT (either bidirectional or polymorphic) or VF. RyR2 mutations were found in 14 (47%) of them. During exercise test, 24 (80%) patients had VT. Clinical and genetic investigation of 118 family members revealed an additional cohort of 13 CPVT patients, of whom 9 had VT of VF during exercise test and 9 carried RyR2 mutations. In the 23 RyR2 mutation carriers (CPVT1 patients consisting of 14 probands and 9 cohort patients), first syncope occurred during childhood (mean age, 8 ± 2 years). Syncopal episodes were much more prevalent among the males (11 of 13) than among the females (2 of 10). The 20 nongenotyped subjects differed from the CPVT1 patients in that they were mostly females (18/20) and their first syncope occurred at the relatively late age of 20 ± 12 years. Programmed electrical stimulation was performed in 21 patients but had a negligible contribution to the diagnosis of CPVT.

All 39 clinically affected patients were treated with beta-receptor blockers and followed for about 4 years. Eighteen (46%) patients had VT or VF while treated. An implantable cardioverter defibrillator (ICD) was implanted in 12 patients, of whom 6 received appropriate shocks during a 2-year follow-up. There was a high juvenile sudden cardiac death rate among CPVT patients (19 of 148 subjects) with no difference between genotyped and nongenotyped subjects.

M. M. Scheinman and J. N. Weiss: Of great concern is the possibility of “resistance” to beta-blocker therapy. In younger patients, patient compliance is a great issue even when dealing with potentially life-threatening arrhythmias. This has certainly been our experience with children of the long QT syndrome.

As in several other primary electrical diseases, the penetrance of CPVT1 was not complete. In the report by Priori et al,11 4 (17%) of the CPVT1 subjects were silent gene carriers. Incomplete penetrance in CPVT1 patients was later confirmed by others.12

Thus, the Priori et al report11 added several important clinical observations to those of Leenhardt et al5: (1) about one-half of the symptomatic autosomal-dominant CPVTs are due to RyR2 mutations, while the genetic
basis for the other half is currently unknown; (2) syncope in CPVT may be due to VF (in addition to bidirectional and polymorphic VT); (3) the first syncope may occur relatively late in the third decade of life; (4) males are at an increased risk for syncope; (5) beta-blockers are only partially effective; and (6) a significant minority requires ICDs.

Relatively little is known about the nongenotyped patients. Interestingly, most of them were female but the reason for this gender inequality is not known. There is a definite genetic heterogeneity in CPVT1. In a study by Tester et al of 11 patients who were referred for genetic testing with a clinical diagnosis of CPVT, 3 had mutations in the KCNJ2 gene (associated with LQT7), a gene not tested in the Priori article.

Sinus Bradycardia in CPVT

Sinus bradycardia was reported by Leenhardt et al in 1995 but was not mentioned by Priori et al. This feature was later described in 29 nongenotyped Japanese CPVT patients and in a European series of 12 families carrying 13 different RyR2 missense mutations. Mutation carriers in these series had a resting heart rate that was 20 beats/min lower than that of age- and gender-matched controls, and 12 beats/min lower than that of their nonaffected family members. The heart rate of affected males was significantly lower than that of females (compared to matched controls). The reason for bradycardia is unknown. Postma et al hypothesize that it may result from impaired Ca$^{2+}$ handling by mutated RyR2 channels in sinoatrial node cells or be mediated by a vagal effect.

Several atypical clinical features of CPVT1 were detected in a family described by Allouis et al, in which 11 subjects carried the G14876A mutation in the RyR2 gene, 10 of whom were symptomatic. Several unusual characteristics were noted, as follows: (1) while four patients on exercise had bidirectional or polymorphic VT, five patients had mostly monomorphic VPCs or short VTs (up to quadruplets); (2) while symptoms (syncope and sudden death) occurred mostly during exercise or emotion, two young patients died suddenly during sleep. One of these patients had never had exercise-related symptoms, while the other (the proband) had experienced several emotion-related syncopal episodes. Based on these findings, the authors recommend a full-scale family screening of each case of sudden cardiac death in a young patient, in order to detect a typical catecholamine-related presentation to facilitate a correct diagnosis.
M. M. Scheinman and J. N. Weiss: Ackerman and colleagues have emphasized the importance of looking for RyR2 mutations in patients with sudden death (forensic autopsy) and in survivors of sudden cardiac death.

**CPVT and Congenital LQTS**

It is obvious that phenotypical CPVT is quite similar to congenital LQTS.16 In fact, 9 of the 30 probands in the Priori series11 were misdiagnosed as “LQTS with normal QT interval”16 because syncope occurred during emotion and exercise. Tester et al17 found RyR2 mutations in 17 of 269 (6%) unrelated, genotype-negative patients referred for LQTS genetic testing. Interestingly, the presentation was near-drowning in five and excitement- or exertion-related syncope in three patients. Six patients had VPCs up to nonsustained VT on exercise test.

Moreover, it is well known that congenital LQT1 syndrome (due to mutations in the KCNQ1 gene) is characterized by swimming-related symptoms. Among 43 patients with swimming-related syncope referred for genetic testing, 28 (65.1%) had LQT1 syndrome and 9 (20.1%) had RyR2 mutation.18 All subjects with the LQT1 syndrome had a high clinical probability, while all those with the CPVT1 genotype had a low clinical probability of LQTS. Molecular autopsy revealed mutations in the RyR2 gene in two other cases of unexplained drowning.12 Catecholamine-related syncope may also occur in the setting of other primary electrical diseases. These include some patients with the congenital LQT2 syndrome, LQT4,19 LQT515 (generally due to polymorphic VT), and LQT7 (due to bidirectional VT).

The clinical manifestations of CPVT2 are similar to those of CPVT1. The largest series to date includes 13 patients from seven nuclear families belonging to a Bedouin tribe, all harboring a homozygous D307H mutation in the CASQ2 gene. Syncope occurred in 12 of them, and an asymptomatic 7-year-old boy developed PVT on the treadmill. Symptoms began at a mean age of 6 ± 3 years (range, 3-12) and were always emotion- or exercise-related. The resting heart rate was quite slow, 64 ± 13 beats/min. The penetrance was complete, as is usually the case in homozygous monogenic diseases. Lethality in untreated subjects is high, and nine children in these seven families died suddenly during the decade before CPVT was diagnosed.

At the last follow-up (personal communication, Dr. Asad Khoury, October 2006), 12 affected families were identified in the tribe, encompassing 25 CPVT2 patients. The follow-up period ranged between 2 and
11 years (mean, 5.2). To date, electrocardiograms and echocardiograms in all patients, and cardiac magnetic resonance imaging in six of them, are normal. Syncope occurred in 13 and seizures in 7 patients, while PVT developed during exercise tests in all 25 patients. All are being treated with beta-receptor blockers (mostly propranolol, 4.8 mg/kg/day). During the follow-up period, 17 (68%) patients remained asymptomatic, including 12 patients who had been symptomatic previously. ICDs were implanted in six patients (the four with syncope and two following the sudden death of a sibling). Four (16%) patients had at least one syncope episode and four (16%) patients died suddenly. Some of these patients are known to have poor compliance, and two of the four who died suddenly had refused ICD implantation.

**Molecular Basis of Calcium-Mediated Arrhythmia**

The following sections review the physiology of excitation-contraction coupling, providing the background for discussing the pathogenesis of calcium-mediated arrhythmia in general and genetically determined CPVT in particular.

**Physiology of Myocardial Calcium Cycling:**
**Excitation-Contraction Coupling and Calcium-Induced Calcium Release**

The process coupling cardiac electrical activity to mechanical activity is referred to as excitation-contraction (E-C) coupling (Fig 2). Calcium (Ca\(^{2+}\)) is an essential ion largely responsible for the E-C coupling mediating cardiac contraction and relaxation.\(^{20-22}\) The diastolic-free Ca\(^{2+}\) concentrations in the extracellular space [Ca\(^{2+}\)]\(_e\), in the cytosolic compartment [Ca\(^{2+}\)]\(_i\), and in the sarcoplasmic reticulum [Ca\(^{2+}\)]\(_{SR}\) are approximately 1, 100, and 1-3 mM, respectively. Therefore, a gradient of \(\times 10^4\) magnitude exists across the sarcolemmal and sarcoplasmic reticulum (SR) membranes, which is of crucial importance for cardiomyocyte contraction and relaxation.\(^{23}\) During systole, [Ca\(^{2+}\)]\(_i\) rapidly rises from \(\sim 100\) nM to \(\sim 1\) \(\mu\)M, allowing Ca\(^{2+}\) binding to troponin C and triggering the cascade of conformational changes leading to sarcomere contraction. Given the enormous buffering capacity (\(\sim \times 100\)) of the cytosolic compartment, as a result of numerous Ca\(^{2+}\) binding proteins such as troponin C, SR Ca\(^{2+}\) pump, myosin, etc, a large amount of Ca\(^{2+}\) (\(\sim 100\) \(\mu\)mol Ca\(^{2+}\) per liter) has to enter the cytosol to allow for the increase in [Ca\(^{2+}\)]\(_i\). There is a small influx of Ca\(^{2+}\) (I\(_{Ca}\)) resulting from the opening of the voltage-dependent Ca\(^{2+}\) channel during cardiac action potential, which triggers a massive release from the SR\(^{24}\) by a mechanism...
called “Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release” (CICR).\textsuperscript{25,26} To accomplish effective CICR, voltage-dependent calcium channels are concentrated in membrane folds (transverse tubules, “T tubules”) in close proximity to clusters of calcium release channels (also called “the ryanodine receptors”) at the terminal cisternae of the SR. Local increase in \([\text{Ca}\textsuperscript{2+}]_i\) leads to the opening of adjacent SR channels, releasing more \text{Ca}\textsuperscript{2+} from the SR to facilitate contraction.

Termination of \text{Ca}\textsuperscript{2+} release and reduction of cytosolic calcium \([\text{Ca}\textsuperscript{2+}]_i\) to the basal level allow \text{Ca}\textsuperscript{2+} dissociation from the myofilaments and mechanical relaxation.\textsuperscript{20,26} \text{Ca}\textsuperscript{2+} is transported out of the cytosol by four different pathways, which differ between species in their relative contribution. In the rabbit, cat, dog, guinea pig, and humans most of the \text{Ca}\textsuperscript{2+} (∼70%) is recycled into the SR via SR \text{Ca}\textsuperscript{2+}-ATPase (SERCA), while ∼28% is extruded from the cell through Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger (NCX), about 1% is removed by the sarcolemmal Ca\textsuperscript{2+}-ATPase, and an additional 1% is transported into the mitochondria.\textsuperscript{25} The balance in the mouse and rat is different: SERCA removes up to 92% of cytosolic calcium, while
NCX Ca\(^{2+}\) accounts for only \(\sim 7\%\).\(^{27}\) The remaining 1% is split between the sarcolemmal Ca\(^{2+}\)-ATPase and the mitochondrial uniporter. The principal regulators of Ca\(^{2+}\) influx and efflux are therefore the L-type calcium channel (LTCC), the SR calcium release channel, NCX, and SERCA. The amount of Ca\(^{2+}\) entering the cell during systole as well as the amount of Ca\(^{2+}\) extruded during diastole is smaller in rodents, a point to be considered when studying human disease in rodent models.\(^{20}\)

**Voltage-Dependent Calcium Channels**

Two kinds of voltage-dependent Ca\(^{2+}\) channels (L- and T-type) open in response to membrane depolarization to mediate calcium current (\(I_{\text{Ca}}\)) where Ca\(^{2+}\) enters the cytosol along with the electrochemical gradient. T-type Ca\(^{2+}\) channels are functionally important in pacemaker tissue, while they are less abundant in the ventricle. The LTCC (also called dihydropyridine receptors) open at higher membrane potential (\(-30 \text{ mV}\) versus \(-60 \text{ mV}\)), have larger conductance and slower inactivation compared to T-type Ca\(^{2+}\) channels, and account for the majority of \(I_{\text{Ca}}\) during action potential.\(^{28,29}\) Inactivation of LTCC primarily occurs by a rise in intracellular Ca\(^{2+}\) and also by a voltage-dependent mechanism. The \(\alpha_1\)-subunit of LTCC possesses the main channel regulatory characteristics: voltage dependency, sites for ligand binding, and phosphorylation. The Ca\(^{2+}\)-dependent inactivation site of the LTCC is located in the carboxyl tail of the \(\alpha_1\)-subunit next to the channel pore and is a target for drugs designed to block channel activity, such as phenylalkylamines (verapamil) and dihydropyridines (nifedipine). During \(\beta\)-adrenergic stimulation, protein kinase A (PKA) phosphorylates LTCC, decreases the threshold potential, and increases \(I_{\text{Ca}}\) by two- to fourfold. Calmodulin-dependent protein kinase (CaMK2) also phosphorylates LTCC on the \(\alpha_1\)-subunit and \(\beta_{2a}\)-subunit, increasing the opening probability.\(^{30}\)

**Sodium-Calcium Exchanger**

The NCX is an electrogenic counter-transporter trading three Na\(^{+}\) ions for one Ca\(^{2+}\) ion, which can operate in two modes (forward Ca\(^{2+}\) extrusion and reverse Ca\(^{2+}\) entry), thereby generating two kinds of currents. The \(I_{\text{Na/Ca}}\) direction and current intensity are determined by the transmembrane concentrations of Ca\(^{2+}\) and Na\(^{+}\), and by membrane potential (\(E_m\)). During the cardiac cycle, the \(I_{\text{Na/Ca}}\) current direction changes twice. The first change occurs quickly after the upstroke of action potential when NCX transports Ca\(^{2+}\) into the cell, creating a net
repolarizing current. The second change occurs after repolarization when NCX removes Ca\(^{2+}\) out of the cytosol, generating a net depolarizing current.\(^{25}\)

The contribution of NCX activity to CICR during cardiac systole is disputable.\(^{31}\) Functioning in reverse mode, NCX increases \([\text{Ca}^{2+}]_i\) and could help trigger CICR. Ca\(^{2+}\) entry through NCX was shown to cause SR Ca\(^{2+}\) release at very high membrane potentials (eg, 70 mV). This mechanism may even allow SR Ca\(^{2+}\) release and contraction, while \(I_{\text{Ca}}\) through LTCC is blocked.\(^{32}\) However, CICR induced by NCX-mediated Ca\(^{2+}\) influx occurs with a 60- to 120-ms delay after phase 0 of the action potential compared to \(\sim 10\) ms when Ca\(^{2+}\) influx occurs through the LTCC. Under prevailing physiological conditions, given a lower membrane potential and lack of confinement of NCX to the transverse tubular system, the contribution of NCX-mediated Ca\(^{2+}\) entry to CICR appears to be quite small.\(^{33,34}\)

NCX does serve as the major Ca\(^{2+}\) transporter out of the cell and hence plays a key role in the relaxation along with SERCA. NCX working in forward mode causes membrane depolarization. Increased NCX activity is a major contributor to diastolic afterdepolarizations and triggered arrhythmia during pathological conditions associated with calcium overload. Overexpression of NCX leads to depletion of SR Ca\(^{2+}\) stores and contractile failure. In this model, adrenergic stimuli generate a large inward current during diastole, which is mediated by NCX functioning in “forward mode” and causes a delayed afterdepolarization (DAD). Such potential may reach the threshold and evoke premature action potential, an electrical event which may propagate into cardiac arrhythmia.\(^{25,35}\)

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**M. M. Scheinman and J. N. Weiss:** The similarity between afterdepolarization triggered ventricular arrhythmias observed with digitalis toxicity as well as in patients with CPVT led Priori and colleagues to suspect abnormalities in Ca\(^{2+}\) metabolism in the genesis of arrhythmias in CPVT. This led in part to the successful discovery of RyR2 as a candidate gene in these patients. Digitalis leads to characteristic arrhythmias due to Na\(^+\)/K\(^+\) ATPase inhibition and genesis of the Ca\(^{2+}\) overload state. This is similar to subjects with impaired Ca\(^{2+}\) handling due to RyR2 or CASQ2 mutations.

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**Sarcoplasmic Reticulum**

The cardiac SR is an intracellular Ca\(^{2+}\) storage organelle that provides most of the Ca\(^{2+}\) needed for contraction (Fig 3). SR surrounds each myofibril and is divided into segments containing a longitudinal part and
terminal cisternae. The junction between the transverse tubule and two cisternae (one on each side) is called a triad.

**FIG 3.** Schematic illustration of the conformational changes occurring in calsequestrin (CASQ) in the course of Ca\(^{2+}\) binding and the resulting modulation of ryanodine channel (RyR) activity. (A) Negatively charged CASQ is a bulky molecule that inhibits the RyR channel. Upon binding of Ca\(^{2+}\), CASQ molecule undergoes stabilization, shrinkage, and polymerization. These changes affect the interaction with RyR, increasing the channel open probability. With even higher SR Ca\(^{2+}\) load, CASQ dissociates from the membrane, disinhibiting the RyR channel. (B) A close-up on calsequestrin. Three-dimensional model of CASQ molecule according to Wang et al.\(^4\) The molecule comprises three thioredoxin-like domains surrounding a negatively charged core created by multiple aspartic and glutamic amino acids. Aspartate 307 substituted to histidine in a human CPVT causing mutation is shown in red. Mutation is assumed to impair the conformational changes occurring in CASQ2 with Ca\(^{2+}\) binding and the ability to regulate RyR2 channel. (C) A close-up on SR Ca\(^{2+}\) channel complex. The channel is composed of four RyR2 polypeptide chains and is associated on the luminal side with triadin (T), junctin (J), and calsequestrin (CASQ2). On the cytosolic side the channel is located in close proximity to L-type Ca\(^{2+}\) channels on T-tubules (not shown), and each chain is capable of binding FKBP12.6, calmodulin (CaM), and being phosphorylated by protein kinase A (PKA) and Ca\(^{2+}\)/calmodulin-dependent protein kinase 2 (CaMK2). In addition, the cytosolic domains have regulatory sites responsible for activation or inhibition by different Ca\(^{2+}\) concentrations. (Color version of figure is available online.)
The cardiac couplon (or junction) is a SR calcium release unit that comprises 10-25 LTCCs organized as a circular cluster around ∼100 SR release channels. Each triad has a couplon on each side of the transverse tubule. About 10,000 couplons are activated during cardiac contraction per ventricular myocyte.24

The SR calcium release channel and the calcium pump (SERCA) are the principal SR membrane proteins (Fig 3). The reuptake of Ca\(^{2+}\) back to the SR is performed by SERCA, which is the most abundant protein in the SR membrane, accounting for ∼35-40% of total protein. The process of Ca\(^{2+}\) pumping against a 1,000- to 10,000-fold concentration gradient involves ATP hydrolysis and exchange of H\(^+\) per Ca\(^{2+}\) ion. SERCA \(K_m(Ca^{2+})\) is ∼300 nM. Therefore, under physiological conditions, the pump reaches its maximal output at systolic \([Ca^{2+}]_i\) of ∼1 μM. Phospholamban is a SR transmembrane protein that normally inhibits SERCA by increasing \(K_m(Ca^{2+})\). Phospholamban phosphorylation by PKA and CaMK2 relieves inhibition, which in turn increases SERCA activity and SR Ca\(^{2+}\) uptake and enhances relaxation.37 The SR contains proteins that are essential for SR Ca\(^{2+}\) storage and the regulation of its release. Calsequestrin is the most abundant SR luminal Ca\(^{2+}\) binding protein, which serves as a Ca\(^{2+}\) ion buffer as well as its sensor to regulate the release through the ryanodine receptor channel.38 The buffering capacity of calsequestrin allows the SR to store up to 20 mM Ca\(^{2+}\), leaving the free Ca\(^{2+}\) concentration at 1 mM.

Calsequestrin is predominantly located within the terminal cisternae.39 The cardiac isoform, CASQ2, is a 399 amino acid protein, which is encoded by a gene of 11 exons located in chromosome 1 (1p11-p13.3). The skeletal and cardiac muscle isoforms (CASQ1, CASQ2, respectively) are encoded by two different genes. CASQ2 can be found in both cardiac and slow-twitch skeletal muscle, whereas CASQ1 is rather restricted to the fast-twitch skeletal muscle. There is a high degree of homology between the different isoforms and between different species, implying that studies on one animal or tissue may be pertinent to others.40 Crystal structure of rabbit skeletal calsequestrin revealed three thioreduxin-like domains and an acidic core, which are presumably stabilized upon Ca\(^{2+}\) binding (Fig 3B41). The fact that calsequestrin contains an excess of 60-70 negatively charged amino acid residues is responsible for its remarkable Ca\(^{2+}\) storage capacity, enabling it to bind ∼40 Ca\(^{2+}\) ions.42,43 CASQ2 interacts with SR membrane proteins, triadin, and junctin through its C-terminal and with other CASQ2 molecules through its N-terminal. Upon increase of \([Ca^{2+}]_{SR}\) and Ca\(^{2+}\) binding, CASQ2 undergoes a conformational change, condenses, and polymerizes (Fig
At $[\text{Ca}^{2+}]_{\text{SR}}$ of $\sim 1$ mM the CASQ2 polymer is stable and anchored to the SR membrane through triadin and junctin. Higher $[\text{Ca}^{2+}]_{\text{SR}}$ concentrations (5-10 mM) cause CASQ2 to dissociate from the SR membrane. Luminal Mg$^{2+}$ competes with Ca$^{2+}$ (with lower affinity) on binding to CASQ2. Hydrogen ions also bind to the acidic residues. During systole, protons counterflow into the SR to compensate for Ca$^{2+}$ efflux, reduce luminal pH, and decrease the Ca$^{2+}$ binding affinity through conformational change and unfolding of the CASQ molecule.

In addition, CASQ2 may undergo glycosylation and phosphorylation, either by casein kinase II present in the SR lumen or by autocatalytic activity in the presence of MgATP. The physiologic role of CASQ2 phosphorylation as well as its ability to phosphorylate other SR proteins is currently unknown. However, it was demonstrated that dephosphorylated CASQ2 may bind to purified ryanosine receptor channels and increase their channel activity.

Other Ca$^{2+}$ binding proteins have been identified. A histidine-rich Ca$^{2+}$ binding protein and sarcalumenin are distributed through SR and may modulate RyR activity in a phosphorylation-dependent manner. Calreticulin is a $\sim 46$ kDa protein found in the smooth muscle and endoplasmic reticulum, where it functions as a chaperone helping in the folding of proteins and glycoproteins. It is highly expressed in the heart during embryonic development but is replaced by calsequestrin after birth.

### The Ryanodine Receptor

The ryanodine receptor traverses the SR membrane from the SR lumen to the cytosol and comprises the core of a Ca$^{2+}$ release channel (Fig 3C). It normally opens to release Ca$^{2+}$ from the SR in response to an increase in the cytosolic Ca$^{2+}$ in the heart or membrane potential in skeletal muscle. Ryanodine, a plant alkaloid, is a specific ligand having a biphasic effect on the channel: it increases Ca$^{2+}$ release at low concentration ($\sim 0.01-10$ μM), whereas higher concentrations (200 μM) have an inhibitory effect.

There are three mammalian isoforms of ryanodine receptor (RyR1 to RyR3) expressed in different tissues and encoded by three different genes having $\sim 70\%$ homology. RyR1 is mostly expressed in the skeletal muscle. RyR3 is associated mainly with the brain but can also be found in other tissues like abdominal organs, skeletal, and smooth muscle. RyR2 is considered to be a cardiac isoform but is expressed in many other cell types, in particular, the brain and the kidney. The skeletal and cardiac isoforms differ in their opening mechanism. RyR1 opening is voltage...
dependent and presumably involves a physical interaction between RyR1 and LTCC but does not require Ca\(^{2+}\) influx to occur. In contrast, Ca\(^{2+}\) entry through LTCC is necessary for activating RyR2 through the CICR mechanism in the heart.\(^{52}\)

The cardiac SR Ca\(^{2+}\) release channel is a huge structure comprising four RyR2 units of 565 kDa each. RyR2 monomer is encoded by a gene located on chromosome 1 (1q42-q43), comprising two principle parts. A large (∼4500 amino acids) N-terminal cytosolic part has multiple binding sites and serves to regulate channel activity (Fig 3C). A smaller C-terminal part (∼500 amino acids) appears to contain the transmembrane domains (most probably six). Each monomer contributes two of these domains to create the channel pore located between the parallel domains of all four RyR2 molecules. The C-terminal part also contains sequences for linking between the RyR2 monomers, and for interaction with CASQ2, triadin, and junctin proteins, responsible for the SR luminal regulation of Ca\(^{2+}\) release.\(^{44,53}\)

RyR2 channel activity is tightly linked to cytosolic calcium. The gating model suggests three functional states. At rest, the channel is closed and can be opened by binding Ca\(^{2+}\) to the low affinity activation site. As [Ca\(^{2+}\)]\(_i\) rises further, Ca\(^{2+}\) binds with higher affinity to an inactivation site, switching the channel to a closed mode. Once [Ca\(^{2+}\)]\(_i\) decreases, Ca\(^{2+}\) first dissociates from the low affinity site and only later from the high affinity site, switching the channel from the closed-inactive state to the resting state. Another model of regulation involves channel adaptation: after rapid activation following [Ca\(^{2+}\)]\(_i\) elevation, the open probability is lowered, presumably to protect from inappropriate release. This partial refractoriness can be overcome by a further increase in [Ca\(^{2+}\)]\(_i\) concentration.\(^{20,54}\)

Luminal Ca\(^{2+}\) affects RyR2 opening by direct and indirect means and thus modulates the amount of Ca\(^{2+}\) released from the SR. At low [Ca\(^{2+}\)]\(_{SR}\), the capacity of I\(_{Ca}\) to generate CICR is low. Increasing [Ca\(^{2+}\)]\(_{SR}\) raises the RyR2 open probability and affects RyR2 sensitivity to [Ca\(^{2+}\)].\(^{55-57}\)

Calsequestrin and transmembrane proteins triadin and junctin participate in Ca\(^{2+}\) sensing and RyR2 regulation. Without CASQ2, triadin and junctin stimulate the channel. CASQ2 serves as a SR-calculator sensor and regulates RyR2 through its interaction with triadin and junctin. At low [Ca\(^{2+}\)]\(_{SR}\) CASQ2 inhibits the channel but, as [Ca\(^{2+}\)]\(_{SR}\) rises, CASQ2 undergoes conformational changes, causing up-regulation of channel activity. At high [Ca\(^{2+}\)]\(_{SR}\) (which may be beyond the physiological
range) CASQ2 polymerizes and dissociates from the complex, further increasing channel activity (Fig. 344,58).

Channel sensitivity to activating stimuli is modulated by the energy state and intracellular Mg$^{2+}$. ATP activates the channel, and Mg$^{2+}$ inhibits it. The cytosolic domains interact with various ligands that regulate its open/close state. Calmodulin has four Ca$^{2+}$ binding sites and binds to RyR in a Ca$^{2+}$-dependent fashion. Each calmodulin molecule binds to a single RyR monomer. Interestingly, while calmodulin decreases the RyR2 open probability at all [Ca$^{2+}$]$_i$ concentrations, its effect on RyR1 is biphasic: increasing its open probability at basal [Ca$^{2+}$]$_i$ but inhibiting it at a concentration higher than 1 μM CaM.24,50,59

FK binding proteins (FKBPs) bind FK-506 and rapamycin, thus preventing the mammalian target of rapamycin complex and forestalling their immunosuppressant and antiproliferative activity. FKB isoforms, FKB12 (calstabin1), and FKBP12.6 (calstabin2) were found to associate with skeletal and cardiac RyR, respectively. FK-binding proteins bind 1:1 to each RyR monomer, stabilize the channel, and decrease its sensitivity to Ca$^{2+}$.60,61

Overexpression of FKBP12.6 in isolated cardiomyocytes showed a decrease in Ca$^{2+}$ flux through RyR2 and an increase in [Ca$^{2+}$]$_{SR}$, implying a decrease in Ca$^{2+}$ release through the RyR2. FKBP12.6-deficient mice suffered from DAD and catecholamine-induced arrhythmia.62 Binding of endogenous FKBP12.6 by FK506 or rapamycin leads to “leaky channels,” an increase in Ca$^{2+}$ sparks and decrease in [Ca$^{2+}$]$_{SR}$.24 Cumulatively these data confirm the stabilizing effect of FKBP12.6 on the RYR channel. Sorcin, a small Ca$^{2+}$ binding protein localized next to the T-tubules, has an inhibitory effect on RyR2, resembling FKBP12.6. This effect is attenuated by sorcin phosphorylation with PKA.63,64

Ryanodine receptor is regulated by phosphorylation and dephosphorylation (with kinases and phosphatases, respectively). Marks65 showed that PKA phosphorylation at Ser$^{2809}$ sensitizes the channel to [Ca$^{2+}$]$_i$ by dissociating FKBP12.6.61 Other researchers disputed these findings66 and suggested that PKA effect on single channel activity is dependent on [Ca$^{2+}$] concentration.67,68 Furthermore, PKA phosphorylates additional key players in E-C coupling, increasing LTCC current and SERCA activity, changes which eventually result in greater Ca$^{2+}$ stores and release from the SR.24

Ca$^{2+}$/calmodulin-dependent protein kinase 2δ (CaMK2δ) is the major cardiac isoform of CaMK, which phosphorylates RyR2, PLB, LTCC, as well as other sarcolemmal channels. Ca$^{2+}$ binds to CaMK2 and activates
kinase activity but the exact location and physiological significance of CaMK2 phosphorylation of RyR2 are still disputed.30,69,70

**Effects of β-Adrenergic Stimulation**

Sympathetic stimulation via β-adrenergic receptor facilitates contraction (inotropic effect) and relaxation (lusitropic effect). Ca\(^{2+}\) transients are of relatively short duration and high amplitude with rapid ascent and decline. The β-adrenergic receptor receptor activates adenylate cyclase through Gs protein, inducing cyclic AMP synthesis. Protein kinase A (PKA) is then activated to phosphorylate target proteins, mediating the sympathetic effects on contractility and metabolism. Phosphorylation of LTCC increases I\(_{\text{Ca}}\). Phospholamban phosphorylation increases Ca\(^{2+}\) uptake into the SR enhancing relaxation and increasing [Ca\(^{2+}\)]\(_{\text{SR}}\). RyR2 phosphorylation leads to FKBP12.6 dissociation, to facilitate Ca\(^{2+}\) release. Cumulatively these mechanisms result in increased RyR2 open probability, thereby explaining increased predisposition to Ca\(^{2+}\)-induced arrhythmia during sympathetic stimulation.54,61

**Calcium Sparks, Waves, and Transients**

The changes in intracellular calcium during every contraction and between the contractions can be visualized and measured using fluorescence probes. The ascent and descent in [Ca\(^{2+}\)]\(_i\) during a cardiac cycle is called Ca\(^{2+}\) transient.71 An elementary unit of Ca\(^{2+}\) release from the SR, generated by Ca\(^{2+}\) influx from a single LTCC and adjacent RyRs, is regarded as calcium spark.72 Ca\(^{2+}\) spark may be visualized using a laser scanning confocal microscope as a single 2- to 4-pA ion current of \(-30\)-ms duration, having an \(-1.5\) \(\mu\)m circular radius.73 Reasonable sparks are produced by 10-200 functionally coupled RYR channels. At negative membrane potentials (\(-40\) mV), a spark may be generated by the opening of a single LTCC, triggering four to six ryanodine receptors. With the spread of the action potential, a rise in local [Ca\(^{2+}\)]\(_i\) up to 10 \(\mu\)M in the vicinity of RyR2 on the terminal cisternae initiates \(10^3\)-10\(^6\) simultaneous sparks over the cardiomyocyte, converging into a Ca\(^{2+}\) transient. During diastole, individual sparks are observed at a low rate of \(-100\) sparks/cell/s (estimated single-channel opening probability of 0.0001/s) and lead to local increases in [Ca\(^{2+}\)]\(_i\) up to \(-200\) nM but are stochastic and do not suffice to generate Ca\(^{2+}\) transient. In states of calcium overload the diastolic spark rate and SR channel sensitivity to cytosolic calcium increase. Some of these events cause sufficient [Ca\(^{2+}\)]\(_i\) elevation to propagate in cytosol in waves, a pathologic phenomenon called Ca\(^{2+}\) wave.24,71
A subpopulation of RyRs is spread over the SR membrane unrelated to T-tubules. These “rogue” RyRs can open without anatomic or functional relationship to the junctional clusters of RyRs. Calcium release from these channels is uncoordinated and insufficient to generate sparks or significantly contribute to CICR. However, it may be involved in diastolic Ca\(^{2+}\) leak, cause SR [Ca\(^{2+}\)] depletion, and participate in the pathogenesis of arrhythmia and contractile dysfunction.\(^{68}\)

**Triggered Arrhythmia and Delayed Afterdepolarization**

Triggered activity is an abnormal depolarization that follows a normal impulse. Early afterdepolarization occurs during the plateau or late repolarization phases of an action potential, while DAD is a late event, initiated after previous action potential has ended.\(^{51}\)

Prolongation of action potential duration, as occurs in hypokalemia and long QT syndrome, can lead to the generation of early afterdepolarization. Calcium overload increasing the RyR open probability and causing diastolic calcium waves is the principal cause of DADs.\(^{74,75}\) An increase in diastolic [Ca\(^{2+}\)]\(_i\) is thought to cause DAD by activating a Ca\(^{2+}\)-dependent inward current (I\(_{ti}\)), which is heterogeneous. The majority (>90%) of I\(_{ti}\) consists of I\(_{NCX}\) in the Ca\(^{2+}\) extrusion mode. Ca\(^{2+}\)-activated Cl\(^-\) current may contribute minimally (<10%). An additional current may be carried by a Ca\(^{2+}\) activated nonselective cation current.\(^{76-78}\)

DAD needs to reach threshold potential to evoke a premature beat to initiate the arrhythmia. Considering the cytosolic buffering capacity,\(^{74}\) it is estimated that 30-40 \(\mu\)mol/l of cytosolic calcium or a transient of 424 nM amplitude will be required to evoke an action potential in caffeine-treated myocytes. In the intact heart, I\(_{ti}\) causes a smaller change in membrane potential due to a higher passive outward current, making it less susceptible to arrhythmia triggered by calcium overload.\(^{75}\)

**Inositol Phosphate-Sensitive Channels and Atrial Arrhythmia.** Inositol 1,4,5-trisphosphate receptor (IP\(_3\)R), a Ca\(^{2+}\)-release channel located on the endoplasmic reticulum, is responsible for Ca\(^{2+}\) release from intracellular stores in nonmuscle cells and in smooth muscle.\(^{79}\) All three muscle cell types express IP\(_3\)R either on the SR or on the perinuclear membrane. The channel agonist IP\(_3\) is generated by phospholipase C cleavage of membrane phosphatidilinositol\(^{4,5}\) bisphosphate (PIP\(_2\)) into IP\(_3\) and 1,2-diacylglycerol.\(^{80,81}\) IP\(_3\)R has a homotetrameric structure similar to the RyR family, and each monomer is \(\sim\)300 kDa. Three IP\(_3\)R isoforms are known: Type 1, which is found in neurons, smooth muscle, and in the heart conducting system; Type 2, which is 69% identical to type 1, is
expressed in the atria and is unique to ventricular myocytes; Type 3, which is 64% identical to type 1, is selectively expressed in the conduction system. IP$_3$R ion conductance is lower by ~50% compared to RyRs. Accordingly, IP$_3$-induced Ca$^{2+}$ sparks have a lower amplitude and longer rise and descent times.$^{82}$

In smooth muscle IP$_3$-induced Ca$^{2+}$ release is an important mechanism contributing to contraction and possibly Ca$^{2+}$ waves and oscillations. The IP$_3$R blocker, heparin, inhibits IP$_3$-induced contraction. In the heart, IP$_3$R is expressed in high levels during embryonic life, where it is assumed to participate in cardiomyocyte differentiation and proliferation but is decreased after birth. In the ventricular myocyte there are about 50-fold less IP$_3$Rs than RyR2s, and their location is mainly in the perinuclear envelope and not in the subsarcolemmal SR. The role of IP$_3$R in E-C coupling in skeletal and cardiac myocytes appears to be negligible. High concentrations of IP$_3$ can initiate SR Ca$^{2+}$ release and contraction in rat ventricular myocyte, yet the Ca$^{2+}$ transients are much smaller and slower compared to those initiated by CICR. It has recently been shown that SR and nuclear envelope are interconnected, ensuring uniformity of Ca$^{2+}$ concentration and release.$^{83}$ Therefore, although calcium release through IP$_3$R does not participate in CICR, it may have a modulator effect on E-C coupling in ventricular myocytes.

The amount of IP$_3$R2 is 6-10 times higher in the atria than in the ventricle. Some of the receptors are located in the SR next to RyR and adjacent to the IP$_3$ generation sites on the sarcolemma. These IP$_3$ receptors can contribute to E-C coupling, cause Ca$^{2+}$ release, and increase contractility.$^{84}$ Pathological conditions, such as ischemia and heart failure, are associated with increased IP$_3$ generation through phospholipase C activation. While IP$_3$R activity is apparently not affected by Ca$^{2+}$ released from the SR via RyR2, the RyR2 is influenced by Ca$^{2+}$ released via neighboring IP$_3$R (through CICR). It is believed that IP$_3$-induced Ca$^{2+}$ release in the atria can affect ion channel activity (eg, LTCC, NCX, and Ca$^{2+}$-dependent Cl$^-$ channel) and participate in pathogenesis of atrial arrhythmias including fibrillation.

**Arrhythmia and Cardiac Glycosides.** The enzyme Na,K-ATPase (Na pump) is an ubiquitous plasma membrane protein that uses energy from ATP to extrude Na$^+$ from and transport K$^+$ into the cell. Cardiac glycosides, such as digoxin and ouabain, belong to the family of cardiotonic steroids, having in common a high-affinity binding to the cardiac Na pump. This binding results in partial inhibition of the enzyme,$^{85,86}$ leading to intracellular accumulation of Na$^+$. The rise in Na$^+$(i) initially reduces Ca$^{2+}$ efflux through the NCX, thereby increasing
cytoplasmic and sarcoplasmic $\text{Ca}^{2+}$. This rise in sarcoplasmic $\text{Ca}^{2+}$ may reach a critical level, resulting in secondary spontaneous and synchronous release of $\text{Ca}^{2+}$ from the SR during diastole.\textsuperscript{75} The rise in $\text{Ca}^{2+}$ triggers an $I_{\text{i}}$ current responsible for the DADs, which manifest as oscillatory membrane potentials and can induce an arrhythmia.\textsuperscript{87} In fact, digitalis intoxication is the most prevalent cause of DAD-induced triggered activity and typically manifests itself as monomorphic or bidirectional tachycardia.\textsuperscript{76}

**Arrhythmia in Heart Failure**

There is ample evidence for reduced SR $\text{Ca}^{2+}$ content in heart failure, both in animal and in human hearts,\textsuperscript{88-91} ascribed to down-regulation of SR-$\text{Ca}^{2+}$ ATPase (SERCA2a),\textsuperscript{92,93} up-regulation of the inhibitory function of phospholamban on SERCA2a,\textsuperscript{94} increased expression and function of NCX,\textsuperscript{95} and enhanced diastolic SR $\text{Ca}^{2+}$ leak.\textsuperscript{96} Despite decreased $[\text{Ca}^{2+}]_{\text{SR}}$ and RYR2 protein expression, the release channels have an increased open-probability increasing the spark rate and further depleting the SR calcium stores.\textsuperscript{97}

Heart failure is associated with action potential prolongation, elevated cytosolic $\text{Na}^+$, and increased calcium entry via NCX, apparently during systole. The E-C coupling and the calcium transients are reduced in heart failure models.\textsuperscript{98} The combination of increased NCX and decreased SERCA2a function leads to greater $\text{Ca}^{2+}$ extrusion from the cytosol and lower SR $\text{Ca}^{2+}$ content.\textsuperscript{95} Smaller $\text{Ca}^{2+}$ transients contribute to systolic dysfunction in heart failure. Other changes may be compensatory. Interestingly, a link appears to exist between a markedly decreased SERCA2a function and diastolic dysfunction.\textsuperscript{99}

The mechanism of the enhanced diastolic SR $\text{Ca}^{2+}$ leak is hotly debated. According to the hypothesis by Marks et al.,\textsuperscript{51} heart failure is a hyper-adrenergic state resulting in hyper-phosphorylation of RyR2 by protein kinase A (PKA). This results in partial dissociation of FKBP12.6 from the RyR2, increasing the open probability of the channel and allowing diastolic $\text{Ca}^{2+}$ leak from the SR. Treatment with beta-adrenergic blockers reduced PKA phosphorylation, restored FKBP12.6 binding, and normalized channel function in lipid bilayers and myocardial strips from experimental and human heart failure.\textsuperscript{94,100} Inactivation of phosphodies-terase 4D3, a component of RYR2 complex, resulted in hyper-phosphorylated channel, dilated cardiomyopathy, and arrhythmia.\textsuperscript{101} Heart failure and arrhythmia were attenuated in mice by expressing RyR2, which could not be phosphorylated, due to better FKBP12.6 binding and channel stability.\textsuperscript{101,102}
However, other investigators have failed to reproduce several key aspects of this hypothesis\textsuperscript{52,103,104} and alternative hypotheses have been proposed.\textsuperscript{105,106}

Whatever the mechanism, there is no question regarding the role of the diastolic Ca\textsuperscript{2+} leak in the generation of cardiac arrhythmias in heart failure patients. In nonischemic heart failure, arrhythmias are mainly due to a non-reentrant mechanism, particularly DAD-induced triggered activity.\textsuperscript{25,107,108} The increased inward mode NCX function\textsuperscript{95,109} is coupled with a significantly reduced inward rectifier current (Ik\textsubscript{1}),\textsuperscript{95} which augments It\textsubscript{i}-induced membrane depolarization.\textsuperscript{25} Therefore, there is an increased probability that a resulting DAD will trigger an arrhythmogenic action potential.

**Catecholamine-Induced Ventricular Tachyarrhythmia**

To date, there are 67 known mutations in RYR2 causing CPVT1 and seven CASQ2 mutations reported to cause CPVT2. All RYR2 mutations, except one, are localized within four well-defined protein domains (Fig 4A), although other regions may be implicated in RYR1 in association with malignant hyperthermia and central core disease. As far as one can extrapolate, currently no specific localization is apparent for CASQ2 mutations, although Arg33 might constitute a hot spot for mutagenesis.
The following section discusses how the molecular mechanisms elucidated through genetic studies contribute towards our understanding of the pathogenesis and mode of inheritance of CPVT.

The autosomal-dominant form of CPVT is caused by missense mutations in RyR2, which are considered to confer an abnormal “gain of function.” While the precise molecular mechanism has not been characterized for most of the mutations, they are assumed to cause spontaneous calcium release due to increased sensitivity to luminal calcium or increased responsiveness to phosphorylation by sympathetic agonists. Marks65 proposed a unifying model linking the mechanism of genetically inherited CPVT with that of ventricular tachyarrhythmia in heart failure. PKA phosphorylation at Ser2809 sensitizes the channel to $[Ca^{2+}]_i$ by dissociating FKBP12.6 from the RyR2 complex.61 Exercise increases sympathetic nervous activity, causing PKA phosphorylation, and dissociates FKBP12.6 from RYR2. Single channels from FKBP12.6-deficient mice demonstrate diastolic $Ca^{2+}$ leak when isolated after exercise but not at rest.62 Programmed electrical stimulation or isoproterenol evokes monophasic action potential alternans and bidirectional VT in these mice.62,110 Wehrens et al62 studied S2246L, R2474S, and R4497C human CPVT mutations in isolated RYR2 channels to propose an intriguing but debatable model, suggesting that mutations increase the open probability of PKA-phosphorylated channels by decreasing their affinity to FKBP12.6. Similar results were obtained with “Finnish” RYR2 CPVT mutations: P2328S, Q4201R, V4652F.111 Structurally modified FKBP12.6 capable of binding to either phosphorylated or mutant RYR2 restored normal gating to the mutationally altered channels.62 A novel 1-4-benzothiazepine, JTV519 (recently termed K201), was synthesized and found to increase the binding affinity of FKBP12.6 to RYR2. The drug stabilized isolated channels expressing CPVT mutations. In FKBP12.6 knockout model JTV519 completely abolished arrhythmia in heterozygous but not homozygous mice, suggesting that FKBP12.6 association with RYR2 is essential for normal E-C coupling and that some FKBP12.6 is necessary for antiarrhythmic drug action.59,110

The role of “FKBP12.6” mechanism in heart failure and CPVT has been disputed by others.112 Jiang et al113 found no abnormality in RYR2 and in particular no change in RyR/FKBP12.6 complexes in canine tachycardia-induced heart failure models and in failing human hearts. HEK293 cells transfected with RYR2$^{R4497C}$ had abnormal calcium oscillations, and R4497C mutant channels had increased activity at low cytosolic $Ca^{2+}$ and increased sensitivity to caffeine.92 In another study, RyR2 mutations linked to CPVT and sudden death markedly increased the occurrence of
store overload-induced calcium release, ie, single RyR2 channels were more sensitive to activation by luminal Ca\(^{2+}\).\(^{114}\) These investigators studied a series of RyR2 mutations: Q4201R and I4867M from the C-terminal region, S2246L and R2474S from the central region, and R176Q(T2504M) and L433P from the N-terminal region, to conclude that CPVT mutations increase channel sensitivity to luminal, but not to cytosolic Ca\(^{2+}\) activation, and do not affect the interaction with FKBP12.6.\(^{105}\) Studies in transfected cardiomyocytes also support an FKBP12.6-independent mechanism.\(^{106}\) Decreased Mg\(^{2+}\)-dependent inhibition, CaMKII-dependent phosphorylation,\(^{115}\) conformational instability, and altered interdomain interaction\(^{116}\) are other possible mechanisms. It could be proposed that distinct mechanisms may underlie the propensity to aberrant Ca\(^{2+}\) release in different sets of ryanodine receptor mutations to cause triggered activity and ventricular tachyarrhythmia.\(^{112}\)

Murine models of human disease are often used to validate mechanisms emerging from in vitro studies. Mice lacking the ryanodine receptor die in utero.\(^{117}\) Knock-in mice carrying either the R176Q or the R4497C human mutations recapitulated the CPVT phenotype including polymorphic VT in vivo as well as DADs and triggered activity in vitro.\(^{118,119}\) Contrary to humans, murine arrhythmia was poorly responsive to β-adrenergic blockade. Importantly, unlike isolated channels, FKBP12.6 binding to RYR2 from SR membrane was not compromised by the mutation, and arrhythmia could not be prevented by K201/JTV519. DADs and triggered activity were abolished by ryanodine.\(^{120}\)

The Gyorke group studied the mechanisms of recessively inherited arrhythmia caused by calsequestrin defects in rat ventricular myocytes. The levels of calsequestrin expression were manipulated using adenovirus transfection carrying CASQ2 or antisense CASQ2 transgene. While cardiomyocytes, partially deficient in CASQ2, had reduced SR Ca\(^{2+}\), Ca\(^{2+}\) transients, and Ca\(^{2+}\) sparks, their channels had faster recovery from inactivation and markedly higher release activity after sympathetic stimulation.\(^{26}\) Arrhythmia was attributed to defective Ca\(^{2+}\)-dependent inactivation of RyR2 channels. Overexpression of CASQ2\(^{D307H}\) protein produced effects comparable to CASQ2 deficiency. Cardiomyocytes displayed diastolic calcium oscillations and delayed afterdepolarizations when stimulated after exposure to isoproterenol. It was concluded that decreased SR storage capacity was responsible for disrupted calcium cycling, which could be restored by a Ca\(^{2+}\) buffer-citrate.\(^{98}\)

Protein studies suggested a somewhat different mechanism: D307H mutation compromised CASQ2 ability to undergo conformational change in response to Ca\(^{2+}\) concentration and reduced its binding to triadin and
The authors interpreted their findings as reduced ability of mutant CASQ2 to regulate RyR2 activity. A compatible mechanism was elucidated in cardiomyocytes transfected with CASQ2 harboring a novel R33Q mutation, causing recessive CPVT. Unlike CASQ2$^{D307H}$, CASQ2$^{R33Q}$ was associated with similar Ca$^{2+}$ binding capacity but lacked the ability to inhibit RYR2 at low luminal [Ca$^{2+}$]. The mutation increased the gain of CICR, resulting in leaky SR but with normal amplitude of cytosolic transients despite reduced SR Ca$^{2+}$ stores.

Truncated calsequestrin protein CASQ2$^{G112+5X}$ could not bind calcium, was incapable of conformational changes, and caused severe depletion of SR calcium and abnormal calcium release when expressed in rat cardiomyocytes (despite the presence of a normal endogenous protein). While these findings suggest a dominant negative mechanism, no clinical phenotype was detected in heterozygous carriers of the mutation. CASQ2$^{L167H}$ was identified alongside CASQ2$^{G112+5X}$ in another CPVT patient with compound heterozygosity. This variant had normal Ca$^{2+}$ binding, slightly depleted SR calcium stores, and reduced spark size but no spontaneous transients or DADs after isoproterenol. Collectively, while implicating either defective Ca$^{2+}$ storage or conformational changes causing abnormal regulation of SR release, in vitro studies did not identify a unifying mechanism by which CASQ2 mutations cause disease.

Transgenic mice overexpressing CASQ2$^{D307H}$ in their hearts had normal cardiac structure and function but minor ultrastructural alterations in the morphology of T-tubules and terminal cisternae. While SR Ca$^{2+}$ stores and I$_{Ca}$ current were unchanged, transgenic cardiomyocytes had smaller Ca$^{2+}$ transients and a higher spark rate compared to wild-type controls. Application of caffeine and isoproterenol resulted in abnormal calcium release, Ca$^{2+}$ waves, and delayed afterdepolarizations in mutant CASQ2 overexpressors. ECG recording in intact animals demonstrated increased prevalence of simple and complex ventricular arrhythmia after the drug challenge.

Knollmann et al$^{123}$ were the first to describe mice deficient in cardiac calsequestrin developed to study CPVT caused by homozygous CASQ2 null-allele mutations. Mice were viable and had lower heart rates but normal ECG and cardiac contractility. Besides lack of CASQ2 and a marked decrease in triadin and junctin, there were no changes in protein levels of RYR2 or SERCA, in NCX function, or LTCC current. There was modest ventricular hypertrophy (10% increase in heart/body weight ratio), which was not associated with myofiber disarray or fibrosis but could be related to expansion of SR visualized on electron microscopy. Like human patients, homozygous mice suffered from premature beats.
and polymorphic VT episodes during exercise or isoproterenol infusion. Catecholamines were less effective in inducing arrhythmia in anesthetized mice. Unlike the results from transfected cardiomyocytes, calcium transients, myocyte contractility, and SR calcium stores were surprisingly well preserved in cells isolated from mutant animals. After isoproterenol application, cardiomyocytes from mutant mice displayed spontaneous transient rises in diastolic Ca$^{2+}$ and after-contractions, as well as a 30% decrease in SR calcium stores, consistent with SR calcium leak. The authors concluded that calsequestrin is not required for contractile function but rather to inhibit/control RYR2 channel activity.

Our group engineered murine models of calsequestrin mutations, expecting to establish an experimental model with maximal genetic similarity to human recessive CPVT. Gene-targeted mice with CASQ2 knockout or D307H CASQ2 human mutations were generated. The human arrhythmic phenotype as well as Ca$^{2+}$ oscillations were recapitulated in homozygous but not heterozygous mice (Fig 1C and D). While homozygous knockouts had (as expected) neither CASQ2 RNA nor protein, CASQ2$^{D307H/D307H}$ had a normal amount of RNA but a very low protein level, suggesting protein deficiency as a unifying mechanism of various CASQ2 mutations causing recessive CPVT. Upregulation of calreticulin and of the RyR2 protein compensated for calsequestrin deficiency and allowed mice to survive and have normal cardiac function at rest. We identified increased expression of RyR2 and loss of channel inhibition by CASQ2 as mechanisms potentially responsible for calcium leak and catecholamine-induced arrhythmia. Arrhythmias responded to Mg, which has RyR2 (as well as LTCC) blocking activity.

Calreticulin is the principal calcium-binding chaperon of endoplasmic reticulum and smooth muscle cells. In addition to participating in muscle contraction, calreticulin has multiple actions including gene expression and protein processing. Cardiac expression during neonatal life is required for heart development but falls to very low levels after birth. Calreticulin-deficient animals die in utero because of cardiac defects. Transgenic mice with cardiac expression during adult life develop dilated cardiomyopathy and conduction system disease characterized by sinus node dysfunction and atrioventricular block. Molecular studies identified decreased expression of connexins 40 and 43 and decreased density of LTCC. One would expect that calreticulin expression through adult life in CASQ2-deficient mice might account for other phenotypic manifestations (cardiomyopathy, conduction system disease), which were indeed found in mice and need to be further characterized in humans.

Table 1 provides the mechanisms responsible for Ca$^{2+}$-mediated
arrhythmia following digitalis intoxication, heart failure, and CPVT. Apparently, there are numerous ways to disrupt calcium uptake and release. Multiple regulatory pathways that evolved to control CICR are responsible for the remarkable stability of the system and its capacity to compensate for various perturbations. It is therefore conceivable that several stimuli need to coincide to dysregulate the calcium release system. The clinical presentation of CPVT requires a synergism between a genetic defect (ie, mutation) and an external stimulus (ie, stress or drug). Sensitizing the SR release channel in ventricular myocytes by caffeine increases the spark rate but quickly depletes the SR of calcium and brings the system to a new steady state with downregulated Ca\textsuperscript{2+} release. Adding an adrenergic agonist for concomitant increase in SERCA activity and SR calcium leads to sustained generation of calcium waves. In a canine ventricle wedge preparation, a combination of isoproterenol and caffeine was required to produce monomorphic or bidirectional VT. An additional extrastimulus was often needed to convert it into polymorphic VT. Likewise, human mutations do not suffice to manifest at rest but create a substrate for arrhythmia following another stressful stimulus. In a computerized model simulating RYR2 gating, diastolic calcium release, cytosolic calcium, and DAD evolution, a disrupted RYR2 luminal Ca\textsuperscript{2+} sensing emerged as a unifying pathway of different RYR2 and CASQ2 mutations.

### TABLE 1. Calcium-mediated arrhythmia

<table>
<thead>
<tr>
<th>Digitalis toxicity (Ca\textsuperscript{2+} overload)</th>
<th>Heart failure</th>
<th>RyR2 mutations</th>
<th>CASQ2</th>
</tr>
</thead>
<tbody>
<tr>
<td>RyR2 channel</td>
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<td>Increased</td>
<td>Structural change</td>
</tr>
<tr>
<td>Calsequestrin</td>
<td>No change</td>
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<td>No change</td>
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<tr>
<td>SR Ca\textsuperscript{2+} stores</td>
<td>Increased</td>
<td>Decreased</td>
<td>No change</td>
</tr>
<tr>
<td>Channel sensitivity to luminal Ca\textsuperscript{2+}</td>
<td>No change</td>
<td>Increased (?)</td>
<td>Increased</td>
</tr>
</tbody>
</table>

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**M. M. Scheinman and J. N. Weiss:** The authors have well described the normal operation of Ca\textsuperscript{2+} handling during the cardiac cycle. This involves complex interactions of a number of gene products. While it is clear that approximately 50% of patients with CPVT have genetic mutations related to RyR2 or CASQ2 genes, it is clear that multiple other genetic influences are involved in Ca\textsuperscript{2+} homeostasis. One gets the impression that we are merely skimming the surface in terms of a complete genetic understanding of CPVT. This represents a fertile area for future study and discovery.
Current and Future Therapies for CPVT

CPVT therapy interferes with E-C coupling and must therefore be compatible with adequate contractility and physiological electrical activity. There is general agreement that beta-receptor blockers are the mainstay of therapy for CPVT. However, inadequate response to beta-receptor blocker therapy, or an “escape” phenomenon, is not uncommon. Recurrence and mortality remain high and many patients require an ICD. High doses of calcium channel blockers showed efficacy in animal models and were useful in some of the human patients intolerant to β-blockers. Combining β-blockers with non-dihydropyridine calcium channel blockers carries a risk of symptomatic bradycardia and is not generally recommended. In a recent study in nongenotyped CPVT patients, this combination was more effective than either drug alone. Therefore, cautious administration in highly symptomatic patients, in particular in the presence of a protective pacemaker-defibrillator, appears justified.

While specifically tailored therapy for a given molecular defect is still a long way off, agents to stabilize the disinhibited cardiac ryanodine receptors may target the common pathway underlying CPVT. Although ineffective in a genetic model of CPVT, JTV519 provided valuable proof of this concept by suppressing the arrhythmia in FKBP12.6-deficient mice. The nonspecific RYR2 blocking effect of Mg²⁺ was rather remarkable in CASQ2 mice and this electrolyte has a proven added value in numerous types of ventricular arrhythmia. We suggest that preserving normal levels of Mg²⁺ and supplementations to increase intracellular Mg²⁺ should be recommended to CPVT patients unless otherwise contraindicated. Because much of the proarrhythmic I_{ti} current that underlies the DADs is mediated by the NCX, the exchanger could also be a potential target for inhibition. Regretfully, NCX inhibitors are usually more effective for the reverse mode activity (Ca²⁺ entry), thus carrying a risk of depleting intracellular Ca²⁺ and suppressing the contractility beyond the potential action against DADs. It is therefore encouraging that novel substances emerge that might suppress I_{ti} and attenuate digitalis-induced arrhythmia without compromising contractility.

Exercise is currently prohibited in CPVT patients. The effect of conditioning, sympathetic denervation, and vagal stimulation should be explored, because these interventions might also have a profound effect on susceptibility to arrhythmia and the quality of life of these arrhythmia-prone individuals.
Implications for Genetic Testing

Reaching a genetic diagnosis, ie, identifying a disease-causing mutation, has profound implications on disease management in the affected individual and his/her family members. Mutations can be identified in about 50% of clinically diagnosed individuals with CPVT. RYR2 is the prevailing candidate gene and should be the first to be screened unless disease transmission is incompatible with autosomal-dominant inheritance. Although the value of RYR2 screening in serial victims of sudden cardiac death (SCD) and negative autopsy is considerably smaller (≈15%), RYR2 remains the most common single genetic cause in this population. The contribution of RYR2 mutation to swimming-induced arrhythmia, sudden infant death, and LQT-like presentation is below 5%.

Every family with this malignant disease tends to have its own “private” mutation, and therefore, examination of the coding sequence appears to be warranted (NCBI-RYR2 website). Because RYR2 is a huge gene, a strategy was developed restricting the screening to <20% of gene length but covering the regions encompassing >95% of the mutations described so far. Such a selective screening would be the best choice in light of budget limitations, in particular, when dealing with sporadic cases and victims of sudden cardiac death.

In a cost-contained environment, screening of CASQ2 is warranted after excluding RYR2, in particular, when CPVT is familial and the history is suggestive, or possibly compatible with recessive inheritance (ie, consanguinous, similar ethnic background, etc). Screening for LQT genes, starting with KCNJ2, may be considered in females with bidirectional/polymorphic VT when negative for RYR2 or CASQ2 mutation.

Identifying a disease-causing mutation confirms and refines the clinical diagnosis to facilitate the most appropriate behavioral counseling and therapy. More importantly, genetic diagnosis creates the possibility of genotyping and risk-stratifying asymptomatic family members. CPVT is one of the rare cases when lifestyle modification and even drug therapy appear to be indicated based on genetic diagnosis in the absence of clinical phenotype, since sudden death is not uncommonly the first presentation of this malignant disease.

M. M. Scheinman and J. N. Weiss: We are indeed greatly indebted to the authors for a superb review of the clinical manifestations underlying genetic mutations as well as a review of Ca\(^{2+}\) homeostasis and how abnormalities in Ca\(^{2+}\) handling lead to life-threatening arrhythmias. This review is up to date and required reading for clinicians as well as basic scientists interested in...
genetic arrhythmia syndromes. Especially appreciated by clinicians are the possible innovative future therapeutic options as well as the eminently practical approach to the genetic study of patients with CPVT.

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