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Review

Catecholaminergic polymorphic ventricular tachycardia: Recent mechanistic insights

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Abstract

Cardiac excitation–contraction coupling occurs by a calcium ion-mediated mechanism in which the signal of action potential is converted into Ca2+ influx into the cardiomyocytes through the sarcolemmal L-type calcium channels. This is followed by Ca2+-induced release of additional Ca2+ ions from the lumen of the sarcoplasmic reticulum into the cytosol via type 2 ryanodine receptors (RyR2). RyR2 channels form large complexes with additional regulatory proteins, including FKBP12.6 and calsequestrin 2 (CASQ2). Catecholamines, released into the body fluids during emotional or physical stress, activate Ca2+-induced Ca2+ release by protein kinase A-mediated phosphorylation of RyR2. Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an insidious, early-onset and highly malignant, inherited disorder characterized by effort-induced ventricular arrhythmias in the absence of structural alterations of the heart. At least some cases of sudden, unexplained death in young individuals may be ascribed to CPVT. Mutations of the RyR2 gene cause autosomal dominant CPVT, while mutations of the CASQ2 gene may cause an autosomal recessive or dominant form of CPVT. The steps of the molecular pathogenesis of CPVT are not entirely clear, but inappropriate “leakiness” of RyR2 channels is thought to play a role; the underlying mechanisms may involve an increase in the basal activity of the RyR2 channel, alterations in its phosphorylation status, a defective interaction of RyR2 with other molecules or ions, such as FKBP12.6, CASQ2, or Mg2+, or its abnormal activation by extra- or intraluminal Ca2+ ions. Beta-adrenergic antagonists have proven to be of value in prevention of arrhythmias in CPVT patients, but occasional treatment failures call for alternative measures. There is great interest at present for the development of novel antiarrhythmic drugs for CPVT, as the same approaches may be applied for treatment of more common forms of life-threatening arrhythmias, such as those arising during ischemia and heart failure.

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1. Introduction

Catecholaminergic polymorphic ventricular tachycardia (CPVT), also known as familial polymorphic ventricular tachycardia (FPVT), is an inherited cardiac arrhythmic disorder showing a highly malignant clinical course in the absence of visible morphological alterations of the heart. Since 2001, molecular genetic studies have indicated that CPVT results from inherited alterations of the cytoplasmic calcium handling of the myocardial cells. Although mutations of two different genes have been convincingly related to pathogenesis of CPVT, exact genotype–phenotype relationships remain far from settled, and marked controversy surrounds the theories on exact molecular pathogenesis of CPVT. CPVT appears to be a significant cause of sudden death at young age, underscoring the
importance of its early diagnosis and prophylactic treatment. In fact, molecular studies have pinpointed attractive novel therapeutic targets for specific treatment of CPVT, and possibly for other more frequent forms of ventricular arrhythmias.

2. CPVT may result in life-threatening arrhythmias

CPVT can be inherited in an autosomal dominant [1–5] or recessive [6] way; in some cases, the exact mode of inheritance could not be entirely assessed [7]. Typically, clinical cardiological examinations, including ECG and cardiac echocardiography, reveal mostly normal findings, and postmortem examinations, when carried out, have not disclosed any significant morphological alterations in the fine structure of the heart [3], with the exception of mild fatty infiltration in a few patients [8]. Minor structural abnormalities may occasionally be found on cardiac echocardiograms [3,8]. The hallmark of the disease comprise ventricular arrhythmias of varying morphology that do not exist under resting conditions but appear only upon physical exercise or catecholamine administration. These arrhythmias are first seen as ventricular premature complexes, later resulting in bigeminy and bidirectional or polymorphic ventricular tachycardia [3,9]. These arrhythmias are not rapid enough to endanger hemodynamics suggesting that the lethal arrhythmia, when occurring, is ventricular fibrillation. In different CPVT families, the average heart rate at which the premature ventricular complexes appear on ECG range from 107 to 133 bpm [8,10,11]. Although infusion of catecholamines may induce similar arrhythmias in these patients, programmed electrical stimuli usually do not [3,9], emphasizing the role of circulating catecholamines in pathogenesis of CPVT. Holter recordings are relatively insensitive in phenotyping of the patients, as abnormalities were reported in only 9–21% of affected individuals [3,8]. In some studies, male sex has been shown to increase the risk of syncope by 4-fold [9], whereas other investigators have not detected such gender difference [4,8].

After identification of the principal causative gene as RyR2 (see below), more exact assessment of the symptomatic structure of established gene carriers became feasible. Estimates for penetrance in this disease range from 25 to 100% [3,4,8,9], with an average of 70 to 80%. Syncope appears to be the first symptom in more than half of the patients [9]. Choi et al. [12] provided evidence for overrepresentation of RyR2 mutation carriers in individuals with swimming-triggered arrhythmia syndromes. Swan et al. [3] and Bauce et al. [8] did not report symptoms to occur before 9 to 11 years of age, but other investigators have identified symptomatic DNA-documented CPVT patients among children aged 2 to 9 years [9]. If untreated, the mortality to CPVT is high, reaching 30–35% by the age of 30 years [3,9].

3. Molecular identification of autosomal dominant and autosomal recessive forms of CPVT

The gene locus corresponding to autosomal dominantly inherited CPVT was initially mapped to chromosome 1q42–q43 in two large Finnish families [3]. Thereafter, three groups simultaneously identified this disease gene as the cardiac ryanodine receptor type 2 or RyR2 [4,5,13]. The clinical features of the probands and families examined by Laatinen et al. [4] and Priori et al. [5] were compatible with the CPVT phenotype, while those reported by Tiso et al. [13] were judged to have arrhythmogenic right ventricular dysplasia (ARVD) type 2 or ARVD2, a subtype differing from other forms of ARVD by occurrence of effort-induced polymorphic ventricular arrhythmias, a 1:1 ratio of affected males and females, and high penetrance. Today it is not clear whether CPVT and ARVD2 represent allelic diseases or partly overlapping forms of the same disease, in particular as the causative mutations cluster in the same regions of the RyR2 gene. Thus, more data on genotype–phenotype relationships in ARVD2 are needed.

Until now, a total of 36 different RyR2 mutations have been reported in patients with CPVT or ARVD2 [4,5,8,9,12–17]. They seem to cluster in three different regions of the RyR2 molecule: the N-terminal region, the middle region corresponding to the domain interacting with FKBP12.6 (also termed calstabin2), and C-terminal region (Fig. 1). Several mutations were found to have arisen as de novo, as they were absent in both parents of the affected probands [5,9]. No clearcut relation between the topology of the mutations and the associated phenotypes has emerged until now, and there is no evidence for an apparent mutational hot spot (particular amino acid) in the RyR2 gene. RyR2 mutations have been found in proportions ranging from 18 to 75% of CPVT probands [8,9,14,15], with an average of about 30–40%. Failures to detect mutant RyR2 alleles may be due to occurrence of large gene rearrangements, promoter alterations or intrinsic mutations of the RyR2 gene, or may reflect genetic heterogeneity in CPVT. In fact, a preliminary report describes a single CPVT patient with a missense mutation of the KCN2 gene [18], the mutations of which were previously shown to cause the multiorgan Andersen syndrome. A large number of RyR2 gene polymorphisms present both in patients and non-affected individuals have also been reported [4,13,15], but there is no evidence for a population-prevalent variant showing documented phenotypic effects.

Another type of CPVT, clearly distinct from the autosomal dominant CPVT, was reported by Lahat et al. [6] in a very large Bedouin family. Pedigree analysis suggested autosomal recessive inheritance. Nine affected children had died at the mean age of 7 years and 12 living children had suffered from recurrent syncope and seizures from the mean age of 6 years onwards. Upon treadmill exercise or isoproterenol infusion, polymorphic ventricular tachyarrhythmias appeared at a mean sinus rate of 110 bpm. After initial mapping of the disease locus to chromosome 1p13–21 [6], the underlying gene was identified as the calsequestrin 2 (CASQ2) gene
All affected individuals in the family were found to be homozygous for the missense mutation D307H, while their parents (obligate heterozygous carriers of the mutant gene) were phenotypically entirely normal [6,19].

Postma et al. [7] reported three missense mutations (R33X, 532+1 G > A and 62delA) of CASQ2 in three CPVT families. The homozygous individuals experienced syncopes from the age of 7 to 11 years, and exercise stress tests revealed similar arrhythmias to those seen in CPVT patients with RyR2 mutations, with an average heart rate threshold of 110 bpm; Holter recordings proved to be useful in unveiling of the arrhythmias [7]. The patients tended to have resting bradycardia, like the Bedouin patients studied by Lahat et al. [6]. There was no significant mutation-related family-to-family variation, with the exception that two out of the 16 heterozygous carriers, both with the R33X mutation, had ventricular arrhythmias upon exercise tests, suggesting the possibility of autosomal dominant inheritance with low penetrance, an oligogenic inheritance or a multifactorial background of the disorder in this case.

Using experimental data from cell-free studies with single-channel recordings on lipid bilayers and from living animal organisms, Marks and collaborators have generated an attractive hypothesis linking RyR2 mutations to calcium channel leak during physical exercise and increased adrenergic activity. It should be emphasized, however, that the proposed mechanism remains controversial and has not

4. How do mutant RyR2 channels result in life-threatening arrhythmias?

Mutant mice lacking RyR2 die during embryonic phase with morphological abnormalities in the heart [20].

Unfortunately, this effectively hampers efforts to generate animal models for autosomal dominant CPVT. In fact, there seems to be a marked controversy surrounding the cellular electrophysiological abnormalities that result from mutant RyR2 channels, although there seems to be a consensus in that mutant RyR2 channels are associated with abnormalities in regulation of calcium release from the lumen of the sarcoplasmic reticulum.

RyR2 occupies a central position in cardiomyocytic excitation–contraction coupling (Fig. 2). Upon opening of the cell membrane L-type (dihydropyridine) calcium channels by the depolarizing action potential, small amounts of calcium ions permeate the cardiomyocyte and trigger a tenfold larger release of calcium ions through the RyR2 channels situated at the sarcoplasmic reticulum (SR); this chain of events is called as calcium-induced calcium release (CICR). Coupling of the functions of the L-type channels and RyR2 channels is facilitated by their close localization at the transverse tubule (T-tubule) system (Fig. 2). The calcium ions released in turn interact with the cardiac contractile proteins and thus initiate the systole. During diastole, the calcium ions are pumped back into the sarcoplasmic reticulum via the SR Ca$^{2+}$-ATPase (SERCA2a) or into the extracellular fluid via the sarcolemmal NCX sodium/calcium exchanger (for recent reviews, see Refs. [21–24]).

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been fully accepted by all scientists. Initially, Marks’ group demonstrated that the RyR2 channel, a tetramer complex consisting of four RyR2 subunits, four FK506 binding proteins (FKBP12.6 or calstabin2) and many other interacting components, is phosphorylated by protein kinase A (PKA) which dissociates FKBP12.6 from the complex [25] (Fig. 2). Dissociation of FKBP12.6 from RyR2 increases the channel open probability and induces subconductance states which may provide substrates for arrhythmia. In heart failure characterized by chronic activation of the adrenergic system, RyR2 channels are hyperphosphorylated resulting in their depletion of FKBP12.6 which in turn makes them “leaky”, i.e., more sensitive to induction of CICR (see above) [6]. In a dog model of experimental heart failure, oral administration of beta-adrenergic blockers orally reversed PKA phosphorylation of RyR2, restored the binding of FKBP12.6 to RyR2 and normalized the RyR2 channel function in lipid bilayers in vitro [26]. These studies were extended to the human patients, too. Using myocardial strips prepared from patients undergoing heart transplantation with or without preceding beta-blocker treatment, Reiken et al. [27] showed that long-term beta-blocker treatment restored PKA phosphorylation of RyR2 as well as the amount of FKBP12.6 in the RyR2 channel complex in failing hearts to the levels found in non-failing hearts.

In subsequent studies, Marks and coworkers in collaboration with our group demonstrated that mutant RyR2 channels typical of CPVT show calcium-releasing features similar to those of the wild type (WT) at resting conditions, simulating the cardiac diastole, but after PKA phosphorylation, simulating sympathetic activation, the mutant channels show a ten-fold increased open probability consistent with a gain-of-function defect [11]. In fact, the RyR2 mutant channels are phosphorylated to the same degree as the wild-type channels, but they show a significantly decreased binding affinity to FKBP12.6 [11]. All RyR2 mutations studied (P2328S, Q4201R, V4653F) yielded similar results. The degree of the gain-of-function defect and “leakiness” of the channels increased from heterotetrameric (WT RyR2 complexed with mutant RyR2 subunits) to homotetrameric (only mutant RyR2 present) channels. An additional role could be played by magnesium ions (Mg^{2+}) (Fig. 2). Thus, PKA-phosphorylated CPVT-mutant RyR2 channels showed increased resistance to inhibition by the channel-stabilizing Mg^{2+} ions, which may also increase the propensity for ventricular tachyarrhythmias and sudden death [11]. It is also of note that RyR2 may be phosphorylated by another kinase, Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), on a site distinct from that for PKA [28], but it is at present not known

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**Fig. 2. Regulation of the RyR2 channel function in cardiac myocytes.** Abbreviations: T—T-tubule, L—L-type of Ca^{2+} channel; E, NE—epinephrine, norepinephrine; βAR—beta-adrenergic receptor; cAMP—cyclic AMP; PKA—protein kinase A; FKBP12.6—calstabin2; TRD—triadin 1; JCN—junctin; CASQ2—calsequestrin 2; SR—sarcoplasmic reticulum; SERCA2a—sarcoplasmic reticulum Ca^{2+}-ATPase; NCX—Na^{+}/Ca^{2+} exchanger.
whether RyR2 mutations result in alterations of this pathway. Collectively, the data suggest that a common denominator in CPVT may be diminished FKBP12.6 levels (and increased resistance to inhibition by Mg\(^{2+}\)) of the RyR2 channel complex during adrenergic activation, thus resulting in increased calcium leak, which in turn would generate inwardly depolarizing membrane currents, delayed afterdepolarizations (DADs) and arrhythmias [11]. Leakiness of RyR2 channels is thus proposed to play a role in both heart failure and CPVT; however, while the WT RyR2 complexes are depleted of FKBP12.6 by chronic PKA hyperphosphorylation in the former, the mutant RyR2 complexes show a reduced affinity to FKBP12.6 during diastole in the latter.

The data of Marks’ group identify molecular targets for intervention. In the absence of an animal model with RyR2 deficiency, studies were carried out in FKBP12.6-deficient (calstabin2\(^{-/-}\)) mice, showing structurally normal hearts and normal ECG at rest, but ventricular arrhythmias and sudden cardiac death upon exercise, similar to those observed in CPVT patients [10]. The data showed that RyR2 channels from calstabin2\(^{-/-}\) mice showed normal functional characteristics under resting conditions, but increased open probability resulting in diastolic calcium leak and DADs under exercise-associated adrenergic stimulus [10]. Importantly, the 1,4-benzodiazepine derivative JTV519, a new cardioprotective agent that was previously shown to reverse PKA-mediated hyperphosphorylation of RyR2 and normalize its leaky function in heart failure [29], was shown to increase the affinity of FKBP12.6 for RyR2 and to prevent cardiac deaths among the heterozygous calstabin2\(^{-/-}\) mice, but interestingly, not the homozygous calstabin2\(^{-/-}\) mice [30]. In parallel experiments using recordings of the CPVT-associated mutant RyR2 channels in lipid bilayers, JTV519 was shown to promote rebinding of FKBP12.6 and to completely normalize the gain-of-function defect of the phosphorylated P2328S RyR2 channels [11]. In summary, stabilization of the cardiac RyR2 channels by novel pharmacologicals may provide a totally new principle to treat life-threatening ventricular arrhythmias due to genetic defects or heart failure.

However, not all investigators seem to totally agree with the above theory. Jiang et al. [31] studied functional properties of the cardiomyocytic calcium-signaling system using single-channel recordings and preparations from exercise-induced tachycardia canine model as well as human hearts. These investigators found that abnormal Ca\(^{2+}\) uptake, rather than Ca\(^{2+}\) release, contributed to depressed and slow Ca\(^{2+}\) transients characteristics of heart failure, and they did not find any evidence for abnormal interaction of phosphorylated RyR2 with FKBP12.6 or RyR2 leakage. Similarly, Li et al. [32] were unable to find any effects of PKA phosphorylation of RyR2 on the RyR2-mediated Ca\(^{2+}\) release from SR when using intact artificially permeabilized ventricular myocytes. The reasons for the seemingly discordant findings in relation those cited above remain obscure, although experimental conditions related to ion concentrations or solubilization techniques may play a role.

The varying nature of RyR2 mutations may also result in controversial findings. The interaction of different types of mutant RyR2 channels with FKBP12.6 was studied by Tiso et al. [33] using yeast two-hybrid system. It was found that CPVT-associated point mutations increased binding of RyR2 channels to FKBP12.6, while ARVD2-associated mutations had the opposite effect [33]; these data even more complicate understanding of the exact relation between CPVT and ARVD2. Furthermore, Thomas et al. [34] report functional heterogeneity among RyR2 mutations associated with sudden death. Three mutants exhibited a gain-of-function phenotype upon caffeine-stimulation of calcium release in human embryonic kidney (HEK293) cells, whereas one particular mutation (L433P) showed a marked reduction of sensitivity to channel activation [34]. It remains to be explored how this would lead to risk of arrhythmia.

George et al. [35] used beating HL-1 cardiomyocytes to examine the functional consequences of RyR2 mutations. These authors were partly able to confirm the findings of Marks et al. in that Ca\(^{2+}\) release was augmented in the cardiomyocytes transfected with the mutant RyR2 cDNAs, as tested by caffeine or isoproterenol stimulation. However, these effects could not be assigned to diminished interaction of mutant RyR2 channels with FKBP12.6, suggesting that there also must be FKBP12.6-independent defects in the function of CPVT-associated RyR2s [36].

Alternative theories have also been proposed by Chen and collaborators. First of all, these investigators have not been able to find evidence for the assumption that PKA phosphorylation at a specific amino acid residue (serine-2808) of the RyR2 subunit dissociates FKBP12.6 from the channel [37]. Second, after expressing the R4496C mutation (mouse counterpart of the human R4497C mutation) in HEK293 cells, they noticed that the mutant channels displayed increased basal channel activity, whether studied by \(^{3}\)H-ryanodine binding, single-channel recordings or Ca\(^{2+}\) imaging techniques [38]. In addition, the same mutation further enhanced sensitivity of RyR2 activation by Ca\(^{2+}\) ions and caffeine. The authors end up with proposing a store-overload-induced Ca\(^{2+}\) release (SOICR) theory, maintaining that spontaneous Ca\(^{2+}\) release from SR occurs in the cardiomyocytes in the absence of membrane depolarization whenever the SR Ca\(^{2+}\) content reaches a critical level; these Ca\(^{2+}\) spillover waves could in turn lead to DADs and triggered arrhythmia [39]. Using HEK293 cells, proposed to serve an adequate model for cardiac cells in this respect, the authors demonstrated that CPVT RyR2 mutations increased the sensitivity of the RyR2 channels to activation by luminal Ca\(^{2+}\) as well as enhanced the basal level of \(^{3}\)H-ryanodine binding. Thus, RyR2 mutations reduce the threshold for SOICR which increases propensity to arrhythmia [39].

At present, it is difficult to reconcile a unifying hypothesis of the molecular pathogenesis of RyR2-mediated CPVT. There seems to be a general agreement on the idea that mutant RyR2 channels are abnormally activated upon sympathetic action, but whether the mechanism involves an
increase in the basal activity of the channel, alterations in the phosphorylation status of the molecule, a defective interaction with stabilizing molecules such as FKBP12.6 or Mg$^{2+}$ or an abnormal activation by extra- or intraluminal Ca$^{2+}$ ions, or by some other hitherto unknown activators, remains to be further studied.

5. Functional consequences of the CASQ2 mutations

Calsequestrin 2 functions as a Ca$^{2+}$ buffering protein in the lumen of SR (for review, see Ref. [40]) (Fig. 2). Overexpression of CASQ2 in transgenic mice was reported to result in cardiac hypertrophy and suppression of CICR, although the storage pool of Ca$^{2+}$ in SR was upregulated [41,42]. CASQ2 is thought to interact with two other SR luminal proteins triadin-1 and junctin, with all three associating into a macromolecular complex with RyR2. Due to its Ca$^{2+}$ binding ability, CASQ2 is presumed to determine the ability of SR to store and release Ca$^{2+}$ in cardiac myocytes and is also considered to be responsible for termination of CICR [43,44].

Until now, two studies have addressed to molecular pathogenesis of autosomal recessive CPVT. Viatchenko-Karpinski et al. [45] introduced the D307H mutation into the CASQ2 cDNA in an adenoviral vector and studied calcium signaling after transfer into adult rat ventricular myocytes. Myocytes expressing the D307H mutant form of CASQ2 showed a reduced Ca$^{2+}$ storing capacity of SR as well as blunted CICR and spontaneous Ca$^{2+}$ sparks. The myocytes also displayed disturbances of membrane potential and signs of DADs after exposure to isoproterenol [45]. Houle et al. [46] expressed the same D307H mutant CASQ2 protein in COS cells and found that its binding to both triadin-1 and junctin was greatly reduced compared to wild-type CASQ2; biophysical studies indicated significant alterations in the tertiary protein structure of CASQ2.

The scanty data thus suggest that at least one particular mutation of CASQ2 results in its marked physicochemical alterations modifying its Ca$^{2+}$ buffering capacity and interaction with other molecules associated with RyR2, and the Ca$^{2+}$-induced Ca$^{2+}$ release may somehow be destabilized as well. It is tempting to speculate that CASQ2 mutations, by altering responsiveness to the Ca$^{2+}$ release channel complex to luminal Ca$^{2+}$, could result in disturbances of SOICR [39]. This mechanism could therefore comprise a common pathophysiological mechanism to at least some of the phenotypic characteristics seen in both autosomal recessive and dominant forms of CPVT.

6. From bench to bedside: implications for targeted treatment of CPVT patients

The very high mortality rate, amounting 30 to 35% by the age of 30 years [3,9,11], calls for novel effective preventive measures. Unfortunately, sudden death may constitute the first manifestation of the disease [8]. As Holter recordings are of limited value, exercise stress test appears to be the method of choice for initial diagnostics, followed by DNA analysis if feasible.

The molecular pathogenesis of RyR2-mediated CPVT underlines the role of increased adrenergic activity as a culprit in triggering of attacks. In fact, administration of beta-adrenergic blocking drugs stands as the standard treatment of CPVT. Leenhardt et al. [1] encountered 10 (48%) fatalities out of 21 untreated patients but only 4 sudden deaths among 38 nadolol-treated patients; the exact molecular type of CPVT, however, was not studied at that time. In the very small non-genotyped material of Sumitomo et al. [47], mortality was 75% (3/4) in mexiletine-treated, 19% (4/21) in beta-blocker treated and 0% (0/3) in calcium-channel blocker treated CPVT patients. Priori et al. [9] gave nadolol, metoprolol or propranolol to 19 RyR2 patients, but 7 (37%) remained symptomatic. Bauce et al. [8] administered beta-blockers to 26 patients with RyR2 mutations. A repeated stress test demonstrated disappearance of symptoms in 17 patients, while in the remaining 9 patients the drug dose was increased. During the mean-follow-up of 6.5 years, not a single patient had syncopal episodes or died suddenly. Anecdotal evidence for favorable effects of beta-blockers have also been reported in several individual CPVT patients [5,13–15], including one acute pediatric case in which intravenous propranolol administration immediately terminated ventricular tachycardia [48]. In summary, beta-blockers are of value in prevention of symptoms and sudden deaths in RyR2-mediated CPVT, but these drugs do certainly not guarantee elimination of risk of fatal events.

Implantable cardioverter-defibrillators (ICDs) have been used in several CPVT patients in order to terminate life-threatening ventricular arrhythmias [3,5,9]. Due to relatively short follow-up times, it is too early to assess the exact benefits and indications for ICD use in CPVT.

Verapamil interacts directly with RyR2, although drug concentrations needed to this interaction in vitro are higher than those needed for binding to L-type calcium channels [49]. Since verapamil is able to inhibit the sarcolemmal L-type of calcium channel initiating steps eventually resulting in CICR, we considered it pertinent to test its acute ability to prevent exercise-induced ventricular arrhythmias in six CPVT patients with RyR2 mutations who all were using long-term beta-blocker therapy [50] (Fig. 3). Verapamil given intravenously reduced the number of isolated and successive premature ventricular complexes by 76%, and these complexes appeared later and at higher heart rate than in the absence of verapamil. In contrast, intravenous administration of magnesium sulphate, tested because of the stabilizing effect of Mg$^{2+}$ ions on the RyR2 channel, did not inhibit the arrhythmias [50]. This novel finding clearly warrants additional studies with inhibitors of the L-type Ca$^{2+}$ channel. It is of some interest that JTV519, the new compound reported to normalize the leaky function of RyR2...
and to prevent arrhythmias and cardiac deaths in calstabin2+/− mice [30], is structurally related to diltiazem, another L-type channel inhibitor.

Data on the efficacy of drug treatment in prevention of events in the autosomal recessive CPVT are limited. Eleven of the 13 patients with the CASQ2 D307H mutation described by Lahat et al. [6] had a complete resolution of symptoms upon propranolol treatment, and the frequency of the syncopal events has decreased in the remaining two; since introduction of this therapy, no patient has died. Of the three pediatric patients homozygous for CASQ2 truncation mutations, two have become asymptomatic on beta-blocker treatment, while one has continuously suffered from stress-induced synapses despite drug treatment [7]. Collectively, the data tend to indicate that beta-blockers are drugs of choice even for CASQ2-type of CPVT patients; data on other alternatives are so far lacking.

7. RyR2 mutations and sudden unexplained death

Autopsy examinations of approximately 10 to 20% of young individuals who succumb unexpectedly and suddenly fail to demonstrate any apparent cause of death. In these instances, cardiac arrhythmogenic disorders are often suspected but only rarely documented due to obvious diagnostic difficulties postmortem. Using molecular genetic methods, Tester et al. [17] examined 49 young cases (age at
death, 1 to 43 years) of sudden unexplained death and identified 7 (14%) cases with RyR2 mutations. The mutations affected conserved amino acid residues and were absent in 200 healthy individuals, supporting their role as disease-causing. The mutant alleles were detected upon screening of only 18 likely candidate exons out of the 105 exons in total present in the RyR2 gene, maintaining the possibility that the real number of RyR2 mutation-positive cases may have been even larger [17]. This finding, if confirmed by other groups and in other populations, may place the RyR2 mutations high on the priority list when genetic causes of sudden unexplained death are sought for.

8. Ryanodine receptor, calsequestrin, what next?

The last 5 years or so have witnessed the discovery of a previously unrecognized pathophysiological cellular mechanism underlying cardiac arrhythmias predisposing to sudden death. Mutations of two interacting proteins, RyR2 and CASQ2, seem to result in inadequately controlled Ca2+ bursts into the sarcoplasm, with concomitant risk of delayed afterdepolarizations and triggered arrhythmia. There are many other components in this intracellular calcium-signaling system that may as well prove to be liable to mutations and associated with risk of arrhythmia, including calstabin2, triadin-1, junctin and SERCA2a. Interestingly, one particular recurrent missense mutation (G406R) of the L-type calcium channel was very recently shown to cause Timothy syndrome, a multisystem disorder characterized by syndactyly, immune deficiency, cognitive abnormalities and lethal arrhythmias [51], indicating that novel arrhythmic phenotypes associated with calcium-signaling pathways are indeed continuously recognized. It is reasonable to expect that the complex interactions and numerous regulators of the SR RyR2 channel will provide additional targets for pharmaceutical industry in attempts to develop novel antiarrhythmic drugs capable of saving lives.

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