Inherited calcium channelopathies in the pathophysiology of arrhythmias

Luigi Venetucci, Marco Denegri, Carlo Napolitano and Silvia G. Priori

Abstract | Regulation of calcium flux in the heart is a key process that affects cardiac excitability and contractility. Degenerative diseases, such as coronary artery disease, have long been recognized to alter the physiology of intracellular calcium regulation, leading to contractile dysfunction or arrhythmias. Since the discovery of the first gene mutation associated with catecholaminergic polymorphic ventricular tachycardia (CPVT) in 2001, a new area of interest in this field has emerged—the genetic abnormalities of key components of the calcium regulatory system. Such anomalies cause a variety of genetic diseases characterized by the development of life-threatening arrhythmias in young individuals. In this Review, we provide an overview of the structural organization and the function of calcium-handling proteins and describe the mechanisms by which mutations determine the clinical phenotype. Firstly, we discuss mutations in the genes encoding the ryanodine receptor 2 (*RYR2*) and calsequestrin 2 (*CASQ2*). These proteins are pivotal to the regulation of calcium release from the sarcoplasmic reticulum, and mutations can cause CPVT. Secondly, we review defects in genes encoding proteins that form the voltage-dependent L-type calcium channel, which regulates calcium entry into myocytes. Mutations in these genes cause various phenotypes, including Timothy syndrome, Brugada syndrome, and early repolarization syndrome. The identification of mutations associated with 'calcium-handling diseases' has led to an improved understanding of the role of calcium in cardiac physiology.

Venetucci, L. *et al. Nat. Rev. Cardiol.* advance online publication 26 June 2012; doi:10.1038/nrcardio.2012.93

Introduction

Inherited arrhythmogenic diseases, also known as cardiac channelopathies, are a group of conditions characterized by distinctive electrocardiographic manifestations, the onset of ventricular arrhythmias, and a risk of sudden cardiac death. In the past decade, remarkable advances have been made in the understanding of the pathophysiology and clinical features of these diseases, with the discovery of mutations in genes encoding cardiac ion channels or their regulatory partners.

Abnormalities in the control of intracellular calcium, so-called calcium handling, are the common denominator of several forms of inherited arrhythmias. Calcium flux in the myocardium has a central role in the conversion of an electrical stimulus into mechanical activation—known as excitation–contraction coupling.[1] Evidence collected in the past 15 years clearly links mutations in genes that encode calcium-handling proteins to inherited arrhythmia syndromes.[2–4] Identification of culprit genes in inherited arrhythmogenic diseases has led to the hope that these conditions could help to identify new strategies for the prevention of sudden death.

In this article, we review the physiology of calcium handling and describe the molecular mechanisms and phenotypes of calcium-related inherited arrhythmias. We also discuss treatment strategies for these conditions. Dysfunctional calcium handling also has an arrhythmogenic role in acquired conditions that can cause sudden cardiac death, such as heart failure and the cardiomyopathies. Such mechanisms are beyond the scope of this article, but we refer readers to some excellent reviews on the topic.[5–7]

Competing interests
The authors declare no competing interests.

Calcium handling in healthy hearts

The highly coordinated opening and closing of voltage-dependent ion channels located in the membrane of cardiac myocytes generates the cardiac action potential. During the plateau phase, opening of voltage-dependent L-type Ca^{2+} channels (hereafter referred to as just 'L-type Ca^{2+} channels') allows the influx of Ca^{2+} in the plasmalemma. This process triggers the calcium transient and induces opening of sarcoplasmic reticulum (SR) Ca^{2+} release channels—the ryanodine receptor 2 (RyR2). This mechanism is called 'calcium-induced calcium release' and is the fundamental link between electrical and mechanical activation in the heart.[1] The Ca^{2+} released from the SR binds to troponin C and induces a series of allosteric changes in the myosin filaments leading to muscle fiber contraction. The subsequent removal of Ca^{2+} is mediated by the concomitant closing of the RyR2 and the action of SR Ca^{2+} ATPase (SERCA) that pumps Ca^{2+} back into the SR stores. This mechanism corresponds to the descending limb of the calcium transient depicted in Figure 1.

Another component of Ca^{2+}-transient termination is the Na^+,Ca^{2+} exchanger (NCX; Figure 1a). The NCX

Molecular Cardiology, IRCCS Fondazione Salvatore Maugeri, Via Maugeri 10/10a, Pavia 27100, Italy (**L. Venetucci**, **M. Denegri**, **C. Napolitano**, **S. G. Priori**).

Correspondence to: S. G. Priori
silvia.priori@fsm.it

Key points

- Ca^{2+} transport in myocardial cells is a key process of cardiac excitability
- A variety of clinical phenotypes can be caused by genetic mutations in genes controlling calcium handling
- Mutations can affect both the 'intracellular' (sarcoplasmic reticulum calcium release) and the 'transmembrane' (Ca^{2+} influx through voltage-dependent L-type calcium channels) components of calcium handling
- Inherited arrhythmias associated with Ca^{2+} dysfunction are often severe and life-threatening conditions
- Risk stratification and therapy for patients with calcium channelopathies can save lives, but several knowledge gaps and uncertainties remain

extrudes one Ca^{2+} ion (two positive charges) for every three Na^{+} ions (three positive charges) taken into the cell. Thus, the NCX removes Ca^{2+} by generating a net inward depolarizing current—the transient inward current (I_{ti}). In physiological conditions, the relative contribution of the two systems to cytosolic Ca^{2+} removal varies between species.[8,9] In humans, SERCA and the NCX remove around 63% and 37% of the Ca^{2+}, respectively.[10] The NCX becomes very important for the removal of Ca^{2+} in conditions characterized by calcium overload, for example in heart failure or in genetic mutations of the *RYR2* gene. In patients with heart failure, SERCA expression is reduced and NCX expression increased; therefore, the balance of calcium removal moves dangerously in favor of the NCX.[10]

The calcium transient is the result, at the whole-cell level, of coordinated 'local' Ca^{2+} release events (Ca^{2+} sparks).[11] These local releases occur at specialized structures—the calcium release units (CRUs; Figure 1a). The CRUs are preferentially localized at the level of the transverse tubules (T-tubules), where the membrane of the SR is juxtaposed to the cellular membrane. One CRU is formed by clusters of RyR2 (spanning the SR membrane) that are in close proximity to the L-type Ca^{2+} channels (on the cell membrane).[12] The number of CRUs recruited for Ca^{2+} release in each cardiac cycle is an important modulator of the amplitude of the systolic Ca^{2+} transient.[13,14] Ultrastructural abnormalities of the SR at the level of T-tubules are often evident in genetically modified animals with molecular manipulation (overexpression), or in genetic abnormalities affecting calcium handling proteins, and can lead to CRU dysfunction.[12,15]

The effects of β-adrenergic stimulation

Activation of the adrenergic nervous system though β-adrenergic receptor stimulation has profound effects on myocardial calcium handling. Adrenergic stimulation, therefore, also has a critical role in the pathophysiology of calcium-mediated arrhythmogenesis and sudden death. Adrenergic activation increases the amount of Ca^{2+} released from the SR, which can be observed by an increase in Ca^{2+}-transient amplitude. Two major events underlie this phenomenon—enhanced amplitude of the L-type calcium channel current (I_{CaL}) and the increase in SR Ca^{2+} content via stimulation of SERCA (Figure 1b).[16]

Activation of the β-adrenergic receptors initiates a cascade of events that concludes with the phosphorylation of crucial components of the calcium-handling system. In this context, two enzymes with phosphorylation activity are the final effectors—protein kinase A (PKA; also known as cAMP-dependent protein kinase)[17] and Ca^{2+}, calmodulin-dependent protein kinase type II (CaMKII).[18] Phosphorylation of the L-type Ca^{2+} channel increases the amplitude of the current. Phosphorylation of cardiac phospholamban removes its SERCA-inhibiting action, leading to an increase in SR Ca^{2+} reuptake. β-Adrenergic stimulation also promotes PKA-dependent and CaMKII-dependent phosphorylation and activation of RyR2,[19,20] but whether this mechanism has a role in the modulation of Ca^{2+} transient amplitude is unclear.[19,21]

Thus, protein phosphorylation is an important mechanism that enables adrenergic activation to increase SR calcium release. In physiological conditions, this response improves myocardial contractility in response to environmental stressors. However, in the setting of cardiac diseases, such as heart failure, the RyR2 channels can be chronically hyperphosphorylated.[22–24] Such hyperphosphorylation is associated with increased channel open probability, leading to diastolic Ca^{2+} leak from the SR. As a consequence, SR Ca^{2+} content and transient amplitude are reduced. Although still debated,[22,23] CaMKII seems to have a prominent role in this abnormal hyperphosphorylation.[24,25] The effects of adrenergic stimulation in the context of mutations causing catecholaminergic polymorphic ventricular tachycardia (CPVT) are described in detail in the following sections.

RYR2 and *CASQ2* channelopathies

CPVT is an inherited disease characterized by the onset of life-threatening arrhythmias during adrenergic stimulation, such as in exercise or acute emotional stress.[26] Two forms of the disease have been described. CPVT1 is an autosomal dominant disease caused by mutations of the gene encoding RyR2 *(RYR2)*.[3,27] CPVT2 is the autosomal recessive form of the disease and is caused by mutations of the gene encoding calsequestrin 2 (*CASQ2*),[4] an SR protein involved in Ca^{2+} release. *RYR2* mutations have also been suggested to be associated with dilated cardiomyopathy,[28] hypertrophic cardiomyopathy,[29] and arrhythmogenic right ventricular cardiomyopathy.[30] However, given the anecdotal nature of these reports, to conclude that *RYR2* mutations cause a broad spectrum of cardiomyopathies seems premature.

Ca^{2+} handing in CPVT

Arrhythmias in CPVT are elicited by Ca^{2+} release events that are not triggered by an action potential and are, therefore, called 'spontaneous calcium releases' (SCRs). Interestingly, SCRs can also be evoked in the presence of acquired conditions such as chronic heart failure or injury from myocardial ischemia, infarction, or reperfusion.[31] SCR begins as a localized event involving a single CRU, but can also diffuse to neighboring CRUs triggering more Ca^{2+} release[32] to produce a cell-wide calcium wave (Figure 2a). The probability that SCR will lead to a calcium wave is influenced by the balance between SR Ca^{2+} content[33] and the concentration of Ca^{2+}

that induces Ca^{2+} release from the SR—the so-called SR calcium threshold.[34] RyR2 function has a pivotal role in controlling the threshold. Any situation that increases RyR2 opening, such as caffeine consumption, decreases the SR threshold,[35] thus facilitating the induction of SCR. By contrast, agents that reduce RyR2 opening, such as the anesthetic tetracaine, increases the threshold[36] and reduces the occurrence of SCR. Several mutations of *RYR2* that are associated with CPVT have been suggested to act by decreasing the SR calcium threshold so that SCR is readily induced.

Arrhythmogenic mechanisms in CPVT

SCR is the fundamental step for arrhythmogenesis in CPVT. When abnormal Ca^{2+} release occurs, cytosolic Ca^{2+} concentration increases and the cell must activate mechanisms to prevent disruption of Ca^{2+} homeostasis and re-establish the physiological diastolic level of Ca^{2+}. As previously described, one of the mechanisms by which Ca^{2+} is extruded from the plasmalemma is the activation of the NCX that exchanges one Ca^{2+} ion for three Na^+ ions thereby generating I_{ti}. This current produces transient membrane depolarizations after completion of the action potential (Figure 2b), known as delayed afterdepolarization (DAD).[37] When DAD amplitude reaches the voltage threshold for Na^+ channel activation, a 'triggered' action potential is generated. Propagation of an action potential to the entire heart generates an extrasystolic beat. When this chain of events becomes repetitive and several DADs reach the threshold for the generation of propagating action potentials, triggered extrasystolic activity is elicited (Figure 2c).

In several studies, mutations of *RYR2*[38,39] or *CASQ2*[40] that cause CPVT have been shown to alter RyR2 function, facilitate the occurrence of SCRs during β-adrenergic stimulation and, in turn, elicit DADs and triggered activity leading to life-threatening arrhythmias.[41] These observations have prompted investigations to define the precise mechanisms by which *RyR2* and *CASQ2* mutations lead to SCR and arrhythmogenesis.

RYR2 *mutations in CPVT*

The RyR2 channel is a homotetramer.[42] Each subunit is formed by 4,967 amino acids and has a long (~4,300 amino acid) N-terminal cytoplasmic domain. This region binds several regulatory proteins including FK506 binding protein 1B (FKBP1B, also known as FKBP12.6), a putative RyR2 stabilizing protein, PKA, and CaMKII. The last 500 amino acids at the C-terminal of RyR2 form the transmembrane segments encircling the channel pore. The number of transmembrane helices present in this region is unclear, but has been suggested to vary between 4 and 10.[42] These transmembrane segments enable interaction between RyR2 and regulatory intracellular proteins, such as junctin and triadin. These two proteins modulate RyR2 function and mediate interactions between RyR2 and calsequestrin 2. The regulation of opening and closing (gating) of the RyR2 channel is unique and complex. Opening is mainly controlled by Ca^{2+} levels at its cytoplasmic and luminal SR

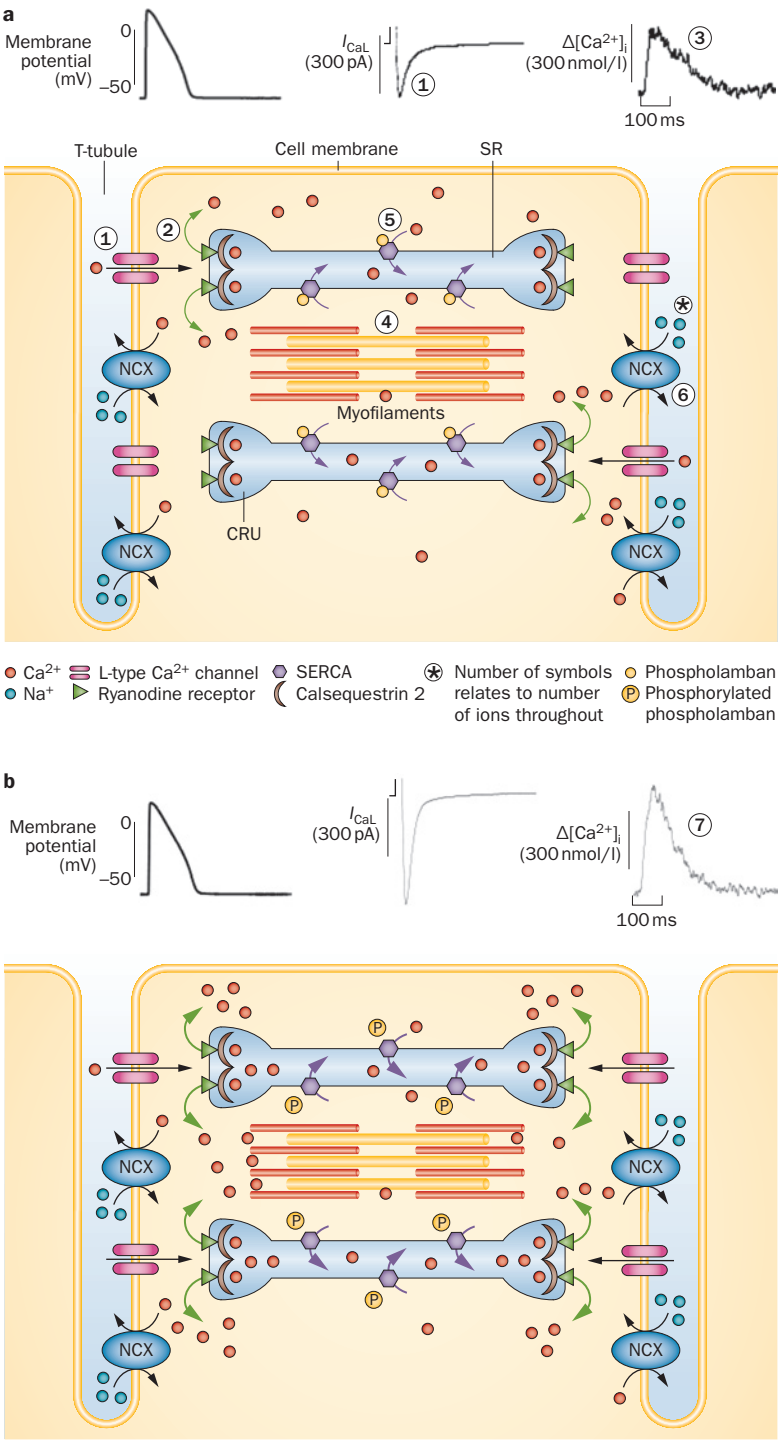

Figure 1 | Calcium-induced calcium release. **a** | Calcium handling. Cardiac depolarization activates the voltage-dependent L-type Ca^{2+} channels (1). Calcium influx activates the CRUs and triggers the release of Ca^{2+} from the SR (2). Summation of Ca^{2+} release at the level of the CRUs produces the systolic calcium transient (3). The transient activates the myofilaments that generate contraction (4). Relaxation is promoted when Ca^{2+} is removed from the cytosol by SERCA (5) and the NCX (6). **b** | β-Adrenergic stimulation increases the amplitude of the calcium transient (7). Stimulation of L-type Ca^{2+} channels increases the number of activated CRUs (green arrows). Stimulation of SERCA, through phospholamban phosphorylation (P), increases reuptake (purple arrows), SR Ca^{2+} concentration, and enhances the amount of Ca^{2+} released by each CRU. Abbreviations: CRUs, calcium release units; I_{CaL}, L-type calcium channel current; NCX, Na^+,Ca^{2+} exchanger; SERCA, SR Ca^{2+} ATPase; SR, sarcoplasmic reticulum.

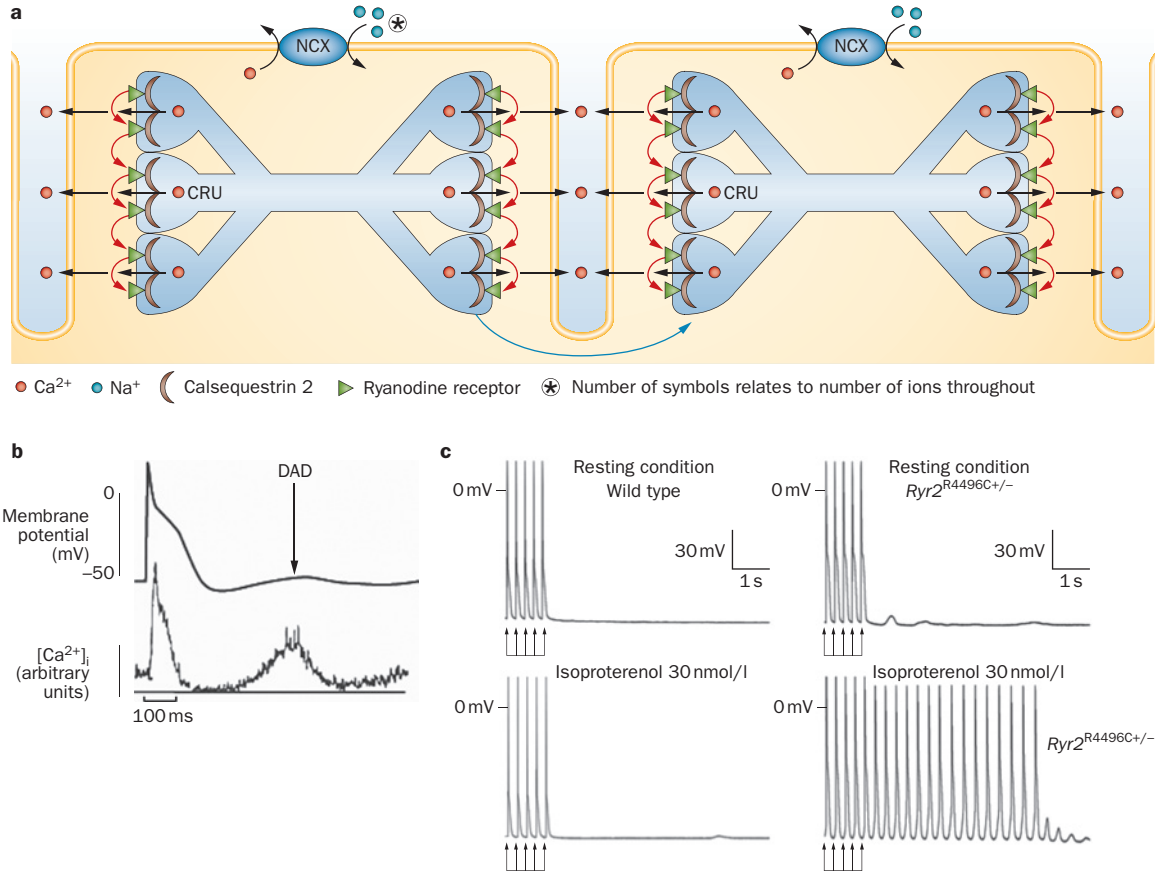

Figure 2 | Spontaneous diastolic Ca^{2+} release and triggered arrhythmias. **a** | In pathological conditions, the sarcoplasmic reticulum can release Ca^{2+} independently from an action potential. Ca^{2+} is released by a pathologically activated CRU, diffuses to and activates neighboring CRUs (red arrows). This process generates a wave that propagates along the cell (blue arrow). Extrusion of excessive cytosolic calcium occurs through NCX activation, which generates an inward current (I_{ti}) and a DAD. **b** | Record of membrane potential (upper trace, action potential) and cytosolic Ca^{2+} (lower trace, transient). The arrow indicates a diastolic spontaneous Ca^{2+} release (lower trace) causing a DAD (upper trace). **c** | Action potentials from ventricular myocytes isolated from a wild-type mouse (left) and a *Ryr2*-R4496C knock-in mouse (right) mimicking catecholaminergic polymorphic ventricular tachycardia. DADs are observed (upper right) in the *Ryr2* mutant at rest. Upon β-adrenergic stimulation, both DADs and triggered activity develop (lower right). Arrows indicate stimulated beats. Abbreviations: CRU, calcium release units; DAD, delayed afterdepolarization; NCX, Na^+,Ca^{2+} exchanger.

sides and is facilitated when the Ca^{2+} concentration at either side increases.[42–45] Several mechanisms have been advocated to explain RyR2 inactivation and calcium-release termination.[46] Currently the most accredited theory is inactivation as a result of a reduction in Ca^{2+} levels in the SR after SR Ca^{2+} release.[8]

Most *RYR2* mutations are missense[47] and tend to be clustered in four regions of the channel[43] that are highly conserved across species (Figure 3). The majority of *RYR2* mutations identified in patients with CPVT cause a gain of function (that is, increased calcium sensitivity).[48] Several groups have studied the mechanism of this gain of function and reached conflicting conclusions. Marks *et al.* suggested that mutant RyR2 exhibits an increased sensitivity to cytosolic Ca^{2+} after PKA phosphorylation.[49,50] Their data also supported the idea that mutations decrease the channel affinity for its accessory protein FKBP1B.[49,50] The same group reported that the binding of FKBP1B to RyR2 stabilizes the closed state of the channel and reduces its sensitivity to cytosolic Ca^{2+}.[49] According to this hypothesis, arrhythmogenesis in CPVT is primarily related to mutation-induced alteration of FKBP1B binding to RyR2.[47,49] During adrenergic stimulation, PKA phosphorylation of RyR2 promotes dissociation of FKBP1B and increases the Ca^{2+} sensitivity and open probability of the channel.[19] The interaction between a RyR2 mutation and the phosphorylated RyR2 channel creates a loop in which mutant RyR2s have a reduced affinity for FKBP1B. This effect is worsened when PKA phosphorylation promotes dissociation of FKBP1B, leading to spontaneous release of calcium from the SR, development of DAD, and arrhythmogenesis.[19,47,49]

This central role of FKBP1B in CPVT has been challenged by other investigators, on the basis of three observations. Firstly, failure to detect differences in the affinity for FKBP1B between mutant and wild-type RyR2.[38,51] Secondly, conflicting or unconfirmed findings for the effect of PKA phosphorylation on dissociation of FKBP1B from RyR2.[52] Thirdly, evidence that most *RYR2*

mutations cause Ca^{2+} leak even in the absence of adrenergic stimulation,[39,53] thus suggesting that adrenergically mediated dissociation of FKBP1B is not necessary for the pathogenesis of CPVT.

An alternative hypothesis to link *RYR2* mutations and arrhythmogenesis in CPVT has been advanced by Chen and co-workers, who demonstrated that the majority of *RYR2* mutations modify the properties of RyR2 so that the channel opens at reduced levels of luminal Ca^{2+}.[39] Interestingly, some of the mutations alter RyR2 response to both luminal and cytosolic Ca^{2+} concentrations.[38,39] The opening of RyR2 at reduced cytosolic Ca^{2+} levels is regarded as a gain of function that, by lowering the SR threshold, promotes SCR and facilitates the development of DADs. This mechanism is called store overload-induced Ca^{2+} release.[38,39]

Yamamoto *et al.* have demonstrated that some *RYR2* mutations cause profound disruption in the 3D conformation of RyR2.[54] They showed that the closed state of the channel is stabilized by tight contacts between the central and N-terminal regions of RyR2. The strength of these interactions was reduced by physiological or pharmacological stimulation, thus leading to channel instability. Reduced 'stickiness' of RyR2 domains is known as 'domain unzipping'. Two other studies have demonstrated that *RYR2* mutations affecting the central domain (between amino acids 2,000 and 2,500) of RyR2 result in domain unzipping and enhance Ca^{2+} sensitivity, thus facilitating spontaneous Ca^{2+} release.[55,56]

The existence of at least three major hypotheses for the mechanism linking *RYR2* mutations to spontaneous SR Ca^{2+} release suggests that a single mechanism is unlikely to operate in all mutations. Rather, as has been observed in other channelopathies such as long QT syndrome or Brugada syndrome, the different CPVT mutations probably lead to similar abnormalities of calcium release and triggered activity through one of the three proposed mechanisms.

The possibility of a loss-of-function effect of *RYR2* mutations has also been suggested. Jiang *et al.* studied *in vitro* an *RyR2* mutation that was previously found in a patient who experienced cardiac arrest in the absence of electrocardiographic or structural abnormalities, and classified this case as 'idiopathic ventricular fibrillation (VF)'.[57] Functional characterization showed a reduced sensitivity to luminal Ca^{2+} (at variance with the increased sensitivity associated with many CPVT mutations).[57] The pathway between this functional abnormality and the clinically observed phenotype remains elusive, thus suggesting that several knowledge gaps need to be filled before RyR2-related arrhythmogenesis can be fully understood.

CASQ2 *mutations in CPVT*

Calsequestrin 2 is a 399 amino acid protein expressed in the SR at the level of the CRUs, where it acts as a moderate affinity (K_d = 1 mmol/l), high-capacity, intra-SR Ca^{2+} buffer and modulates RyR2 activity.[58] The buffering function allows the SR to store large amounts of Ca^{2+} ions near the releasing sites while preserving a low

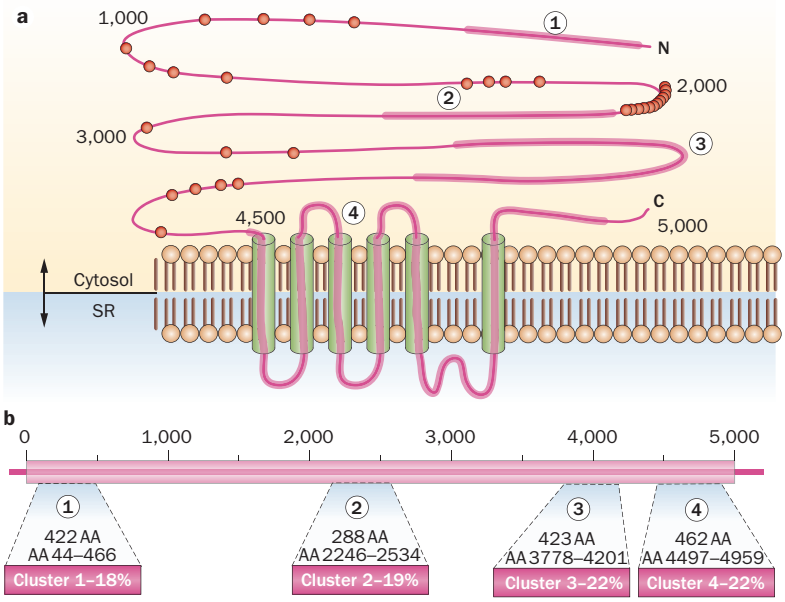

Figure 3 | Structure and mutational clusters of RyR2. **a** | Clusters with frequent mutations are depicted with their location along the protein. Clusters are represented by numbered red lines. Individual mutations outside the canonical clusters are depicted as red dots. **b** | Clusters are represented by boxes, with the AA range of the clusters and the percentage of published mutations for each cluster (AA numbering refers to the human RyR2 protein sequence). The numbers in panel a correspond to the clusters shown in panel b. Abbreviations: AA, amino acid; SR, sarcoplasmic reticulum. Permission obtained from Wolters Kluwer Health © Priori, S. G. & Chen, S. R. *Circ. Res.* **108**, 871–883 (2011).

concentration of free Ca^{2+}. The high Ca^{2+}-binding capacity is generated though the formation of calsequestrin 2 polymers. The presence of negatively charged amino acids at the N-terminal and the C-terminal portions of calsequestrin 2[59] requires the presence of positively charged calcium ions to shield the negative charges and facilitate the polymerization process.[60] Accordingly, at low Ca^{2+} levels (<0.6 mmol/l) calsequestrin 2 is present as a monomer.[51] However, at Ca^{2+} levels between 0.6 mmol/l and 3.0 mmol/l, the protein starts forming dimers by N-terminal interactions. Further increase in SR Ca^{2+} concentration (>3 mmol/l) induces calsequestrin 2 polymers by promoting additional binding through C-terminal interactions.[59]

Calsequestrin 2 is thought to modulate RyR2 via triadin and junctin.[61] The precise mechanism for this regulatory function is still the subject of controversy. Györke *et al.* suggested that calsequestrin 2 is the RyR2 sensor for luminal SR Ca^{2+}.[61] According to their data, at low SR Ca^{2+} levels, calsequestrin 2 is bound to RyR2 and reduces channel opening. When Ca^{2+} concentration increases, however, calsequestrin 2 dissociates from RyR2 and channel opening is facilitated.[61] This model has been challenged by three lines of evidence. First, RyR2 seems to sense luminal Ca^{2+} in the absence of calsequestrin 2.[45,62] Second, Ca^{2+} flux at low levels of SR Ca^{2+} remains normal, with no evidence of enhanced activation of RyR2 in the *Casq2* knock-out mouse.[63] Third, the dissociation of calsequestrin 2 from triadin, junctin, and RyR2 occurs at SR Ca^{2+} concentrations >5 mmol/l,

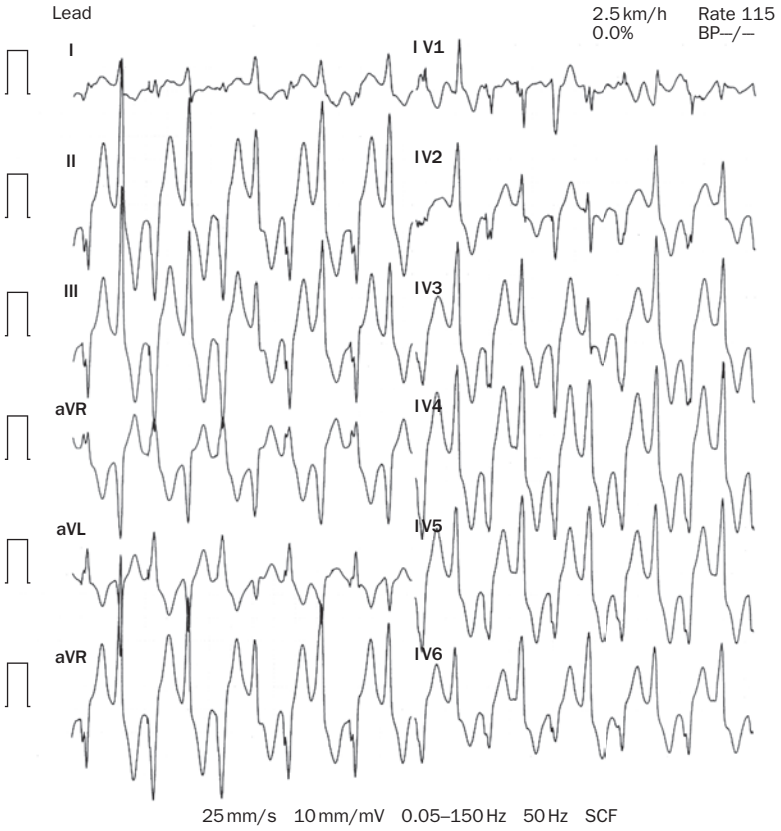

Figure 4 | Episode of bidirectional ventricular tachycardia during an exercise stress test in a patient with catecholaminergic polymorphic ventricular tachycardia. The distinctive alternating QRS axis on a beat-to-beat basis is clearly visible.

which are above physiological SR Ca^{2+} concentrations.[58] Overall, these data do not support the idea that calsequestrin 2 is the RyR2 sensor for luminal calcium, but they do not exclude the possibility that calsequestrin 2 acts as a modulator of RyR2 function.[64]

Homozygous or compound heterozygous mutations in the *CASQ2* gene are required to cause CPVT. A total of 14 *CASQ2* CPVT-related mutations have been described: 10 missense, two nonsense, and two deletions.[47] Two patients with CPVT owing to compound heterozygosis for two different *CASQ2* mutations have been described.[65,66] In-depth functional characterization of two *CASQ2* missense mutations (R33Q and D307H) has shown multiple effects. Reduction in calsequestrin 2 levels owing to increased susceptibility to proteolytic degradation;[67,68] impaired polymerization and buffering capacity;[69–71] and reduced RyR2 binding and loss of RyR2 modulation.[40,72] These mechanisms work in conjunction to generate SCR and DADs,[58] and this chain of events is further exacerbated upon adrenergic activation that induces triggered arrhythmias. Nonsense mutations result in truncated calsequestrin 2 monomers that are unable to polymerize, buffer Ca^{2+}, or bind to RyR2.[61] Interestingly, any reduction in calsequestrin 2 is followed by a parallel reduction in triadin and junctin,[67] suggesting the existence of mechanisms that control the stoichiometry of the three proteins. In agreement with this hypothesis, we showed that the *in vivo* replacement of *CASQ2* by viral gene transfer in a *CASQ2* knock-out mouse model of CPVT not only reconstitutes normal calsequestrin 2 levels, but also normalizes junctin and triadin levels and prevents cardiac arrhythmias.[15] No specific studies of the deletion mutations have been conducted to date.

Proarrhythmic β-adrenergic stimulation

As discussed in the previous paragraphs, even if RyR2 mutations enhance Ca^{2+} sensitivity and lower the SR threshold at rest, arrhythmias occur only during β-adrenergic stimulation. The reasons for this apparent discrepancy are not fully elucidated. Importantly, the relationship between threshold (that is, the SR Ca^{2+} concentration that triggers a release event) and SR Ca^{2+} content should be borne in mind. According to the properties of the Ca^{2+}-release system, in a nonstimulated setting the SR Ca^{2+} content is below the threshold for SCR, thus DADs and arrhythmias cannot occur.[53] Indeed, enhanced RyR2 Ca^{2+} sensitivity and the accompanying reduced threshold also cause a reduction in SR Ca^{2+} content via a phenomenon called 'autoregulation'.[73] After β-adrenergic stimulation, SR Ca^{2+} content increases and reaches the threshold necessary for the occurrence of SCR and DADs. The process of RyR2 channel phosphorylation, owing to the activation of both PKA and CaMKII, has been advocated as the crucial arrhythmogenic step by some investigators.[74] RyR2 phosphorylation enhances Ca^{2+} sensitivity and further lowers the threshold for release.[55,56] This mechanism certainly contributes to arrhythmogenesis, but is not the main proarrhythmic mechanism of β-adrenergic stimulation, because enhancing RyR2 Ca^{2+} sensitivity in isolation is not sufficient to cause SCR.[34]

The proarrhythmic actions of β-adrenergic stimulation are, at least in part, mediated by activation of CaMKII.[75] β-Adrenergic stimulation activates CaMKII through both direct stimulation and an increase in heart rate that promotes CaMKII autophosphorylation and activation.[74,75] CaMKII-mediated phosphorylation increases the amplitude of I_{CaL}, removes the inhibitory effect of cardiac phospholamban on SERCA, and activates RyR2.[25] Stimulation of I_{CaL} and SERCA increases SR Ca^{2+} content, whereas stimulation of RyR2 decreases SR threshold. The combination of these effects facilitates the onset of SCR. The pathophysiological role of CaMKII is confirmed by the observation that inhibition of the kinase prevents arrhythmias in a mouse model of CPVT.[74]

Characteristics and diagnosis of CPVT The first clinical manifestations of CPVT typically occur during childhood.[26] The distinctive clinical feature of this condition is the onset of bidirectional ventricular tachycardia (VT) or polymorphic VT during exertion or acute emotional stress.[76] These arrhythmias can cause syncope or sudden death. Bidirectional VT is characterized by beat-to-beat 180° rotation of the QRS axis on the frontal plane (Figure 4). The diagnosis of CPVT is made when three criteria are met: bidirectional or polymorphic VT triggered by exercise or emotional stress; absence of

structural abnormalities of the heart; and normal QT interval. The only abnormalities that can be, but are not invariably, detected at a standard resting electrocardiogram (ECG) are prominent U waves and sinus bradycardia.[77] These features are considered nonspecific findings and are not, therefore, included in the diagnostic criteria. The diagnosis of CPVT is generally made during an exercise stress test or with 24-h ECG (Holter) monitoring. Epinephrine infusion aimed at inducing the typical bidirectional ventricular tachycardia of CPVT has been proposed as a diagnostic method. However, this strategy seems to have lower sensitivity than the exercise stress test,[78] possibly because vagal withdrawal occurs in addition to adrenergic stimulation during exercise. By contrast, epinephrine injection causes reflex vagal activation (secondary to an increase in blood pressure) that partially counteracts the adrenergic stimuli and might reduce the sensitivity of the test.[79]

Therapeutic approaches to CPVT

Life-long administration of β-blockers is the mainstay of therapy for CPVT, and prevents the onset of arrhythmias in 70–80% of patients.[26,76] In our experience, the nonselective, long-acting β-blocker nadolol provides the highest degree of protection against arrhythmias. Patients who still develop exercise-induced arrhythmias or cannot tolerate β-blockers have an indication for the use of an implantable cardiac defibrillator (ICD). However, the use of ICDs in young patients with CPVT has some limitations, owing to the technical difficulty of implanting the device in children and adolescents and the risk of device-related complications and VT storms over a long period of time.[80] Sympathetic denervation has been proposed as a treatment strategy for patients who are resistant to therapy with β-blockers.[81–83] Case reports on a total of five patients have suggested that this intervention might provide protection from arrhythmias.[81] However, whether these effects endure in the long term is unclear.

Some groups have suggested that the addition of flecainide, a Na^+-channel blocker, to β-blocker therapy prevents arrhythmias in most patients with CPVT refractory to β-blockers alone.[84,85] These reports have generated a great deal of interest in the mechanism of action of flecainide. Watanabe and co-workers suggested that the most-important property for the antiarrhythmic effect of flecainide is the RyR2-blocking activity.[84] These researchers hypothesize that flecainide reduces the duration of channel opening and disrupts the propagation of calcium waves.[86] They concluded that the clinical effect of flecainide is the result of combined RyR2 and Na^+-channel blockade.[84,86] RyR2 blockade reduces the incidence of SCR and DADs, whereas Na^+-channel blockade prevents the triggering of an action potential by any residual DADs (triggered activity). The main objection to these findings stems from the fact that the cytosolic concentration of flecainide needed to achieve sufficient RyR2 blockade is ~25 μmol/l, and the circulating therapeutic concentration of flecainide is 1–2 μmol/l.[87] For a drug that is highly ionized at physiological pH[88] to diffuse through the membrane and reach these cytosolic concentrations (25 μmol/l) *in vivo* is unlikely. We showed that flecainide prevents bidirectional VT in a mouse model of CPVT.[89] In single myocytes, this drug had no significant effects on SCR and DADs, but effectively prevented triggered activity. We concluded that the main antiarrhythmic action of flecainide in CPVT is via its Na^+-channel blocking properties that prevent triggered activity, rather than by RyR2 blockade.[89]

Additional experimental drugs, still not approved for clinical use, have been tested in laboratory (both *in vivo* and *in vitro*) models. A report by Lehnart *et al.* suggested that the 1,4-benzothiazepine derivative K201 (JTV519) reduces RyR2 opening and prevents the onset of arrhythmias in mouse models of CPVT or heart failure.[90] Loughrey and colleagues demonstrated that, in addition to inhibiting RyR2, K201 reduces SERCA activity and I_{CaL}.[91] They concluded that the antiarrhythmic actions of K201 are not exclusively a result of RyR2 inhibition.[91] In 2010, Sedej *et al.* showed that K201 counteracts the arrhythmogenic effects of the cardiac glycoside ouabain in a mouse model of CPVT.[92] Whether this effect is preserved in the absence of digitalis stimulation is unknown. Therefore, the therapeutic role of K201 awaits further confirmation.

L-type Ca^{2+} channelopathies

Structure and function

Three different types of calcium channel are present in the heart: L-type, T-type, and P/Q-type. L-type Ca^{2+} channels are expressed at high levels throughout the heart,[80] and thus far are the only type of Ca^{2+} channel linked to channelopathies.[93] L-type Ca^{2+} channel activation is voltage dependent with maximal activation around 0 mV.[94] Inactivation is dependent on both voltage and Ca^{2+}. The latter mechanism enables the cell to modulate Ca^{2+} influx in response to cytoplasmic Ca^{2+} concentration and prevents Ca^{2+} overload. I_{CaL} has two major functions: modulation of action-potential duration, and triggering the release of Ca^{2+} from the SR.

Cardiac L-type Ca^{2+} channels are composed of one pore-forming subunit α_1, and two accessory subunits $\alpha_2\delta$ and β (Figure 5).[95] Four isoforms of the α_1 subunit exist: α_{1s}, α_{1c}, α_{1d}, and α_{1f}. In the heart, only the α_{1c} and α_{1d} isoforms are expressed. The α_{1c} subunit is the main isoform expressed in the atria and the ventricles, and is also expressed in the nodes. The α_{1d} isoform is predominantly expressed in the sinus and atrioventricular nodes, and to a lesser extent in the atria.[96] Only mutations of the α_{1c} isoform, encoded by the *CACNA1C* gene, have been linked to cardiac channelopathies.[93] The α_{1c} subunit has a structure similar to the voltage-gated Na^+ channels, with four homologous domains composed of six transmembrane segments (Figure 5).[97] These domains are connected to each other through cytoplasmic loops. The loop between domain I and II contains the α interaction domain that binds the β subunit. The distal part of the C-terminal is proteolytically cleaved to form an autoinhibitory peptide[98] that reduces voltage-dependent

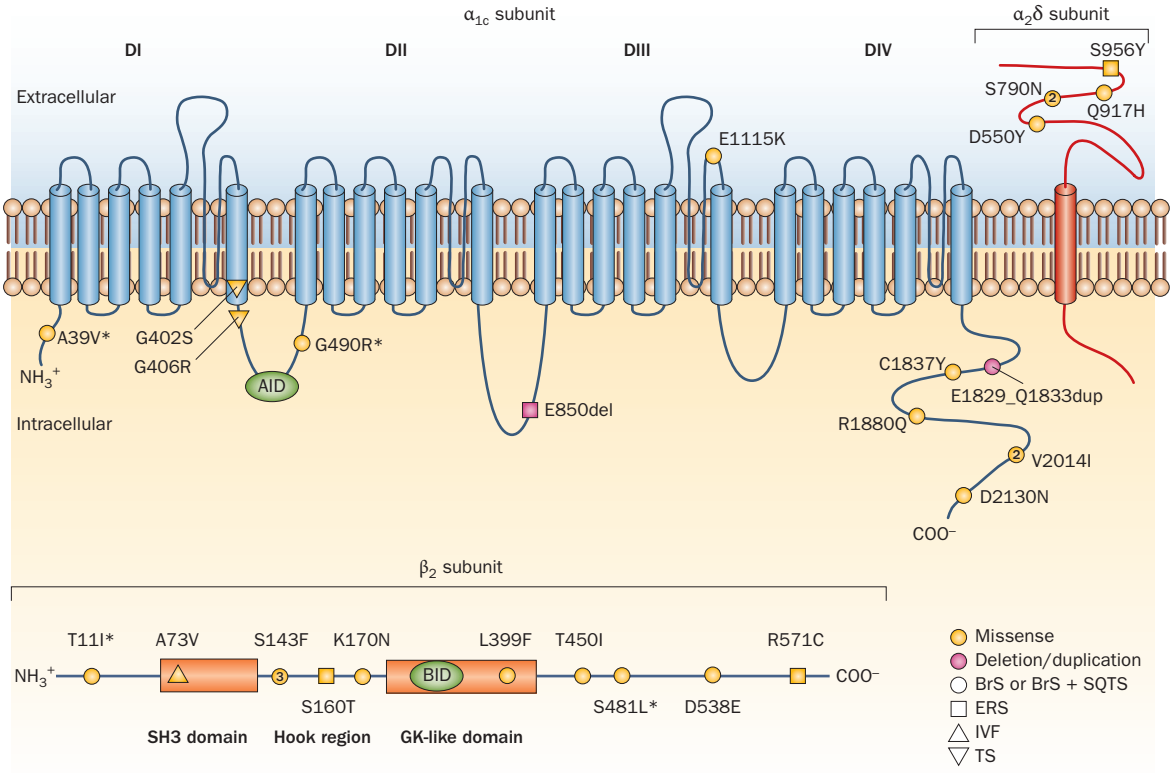

Figure 5 | The L-type Ca^{2+} channel is formed by the assembly of three subunits: α_{1c}, β_2, and $\alpha_2\delta$. The α_{1c} subunit forms the channel pore and is comprised of four homologous domains (DI to DIV) that contain six transmembrane segments. These domains are linked by cytoplasmic loops. The loop between DI and DII contains the binding site for the β_2 subunit (AID). The mutations that cause BrS, ERS, IVF, and SQTS (loss-of-function mutations) are localized in the C-terminal and N-terminal segments and in the cytoplasmic loops. The mutations that cause TS (gain-of-function mutations) are localized in the cytoplasmic loop between DI and DII. The β_2 subunit is completely cytoplasmic and binds to the α_{1c} through the α subunit interaction domain (BID). The $\alpha_2\delta$ subunit is synthesized as single precursor cleaved to generate the two proteins (α_2 and δ subunits). The δ subunit is anchored to the sarcolemma and binds the α_2 subunit. The Figure depicts the reported mutations with different symbols according to the clinical phenotype. If more than one patient or family was identified, the symbols also show the number of probands harboring the mutation. *Previously published variants. Abbreviations: BrS, Brugada syndrome; ERS, early repolarization syndrome; IVF, idiopathic ventricular fibrillation; SQTS, short QT syndrome; TS, Timothy syndrome. Permission obtained from Wolters Kluwer Health © Napolitano, C. & Anzelevitch, C. *Circ. Res.* **108**, 607–618 (2011). Illustration credit Cosmocyte/Ben Smith.

activation. The proximal C-terminal portion of the protein is also very important for the modulation of channel activity. This segment contains amino-acid domains essential to adrenergic modulation, via A kinase anchor protein 15 (AKAP15) and PKA binding,[99] and the calmodulin binding site, which acts as a sensor for Ca^{2+}-dependent inactivation of the channel.[100]

The $\alpha_2\delta$ subunit, encoded by the *CACNA2D1* gene, regulates both activation and inactivation of the L-type Ca^{2+} channel.[101,102] The transcript is translated into a single precursor protein that is post-translationally cleaved to generate the two proteins (α_2 and δ subunits).[100] Deletion of the $\alpha_2\delta$ subunit decreases I_{CaL} amplitude by slowing both channel activation and inactivation.[102]

The β subunit is important for channel trafficking and modulation of I_{CaL} inactivation.[103] Four isoforms are known to exist. β_2, which is encoded by the *CACNB2* gene, is the most abundant in the heart.[104] The β subunit interacts with the cytoplasmic side of the α_{1c} subunit at the level of the loop between domains I and II.

Functional effects

To date, 25 mutations affecting three genes encoding components of the L-type Ca^{2+} channel macromolecular complex (*CACNA1C, CACNB2*, and *CACNA2D1*) have been reported to be associated with Brugada syndrome, Brugada syndrome with short QT duration, short QT syndrome, early repolarization syndrome, and cardiac arrest without ECG abnormalities initially classified as idiopathic VF.[93] Functional studies suggest a loss-of-function effect of these mutations.[93] The behavior of this group of mutations looks similar to what has been described for loss-of-function mutations in the gene encoding the α subunit of the voltage-gated, type V Na^+ channel (*SCN5A*), which can cause various phenotypes—Brugada syndrome, progressive conduction disease, sick sinus syndrome, dilated cardiomyopathy, atrial fibrillation, and overlapping phenotypes.[105,106]

Four *CACNA1C* mutations have been studied *in vitro*.[2,107] The N-terminal missense mutation A39V, which was detected in a patient with Brugada syndrome

and short QT interval, reduces I_{CaL} amplitude by decreasing trafficking of the α_{1c} subunits to the membrane.[107] The distal C-terminal mutation V2041I reduces I_{CaL} amplitude by decreasing channel conductance and by altering I_{CaL} inactivation.[107] The missense mutation G490R and the p.E1829Q1833dup mutation (duplication of five amino acids in the proximal C-terminal) reduce I_{CaL} amplitude, but the mechanism responsible for this reduction is unclear.[2] A loss-of-function mechanism is also associated with *CACNB2* mutations and can be achieved by accelerated voltage-dependent inactivation or reduced I_{CaL} amplitude.[2,108] Of the four missense mutations identified by screening of the *CACNA2D1* gene in patients with Brugada syndrome,[2] none has been expressed *in vitro*. Templin *et al.* identified a missense mutation of *CACNA2D1* (S755T) associated with short QT syndrome.[109] This mutation causes a 70% reduction in Ca^{2+} I_{CaL} amplitude.[109]

From a clinical standpoint, a reduction in I_{CaL} might lead to various phenotypes, but determinants of clinical manifestation remain elusive. The lack of understanding of the clinical consequences associated with the status of 'asymptomatic carrier' of a mutation in the genes encoding subunits of the L-type Ca^{2+} channel complicates the use of genetic testing in clinically unaffected family members. Therefore, recommending a conservative approach in the extension of genetic testing to asymptomatic family members with a normal ECG seems reasonable, until we understand more about the prognostic implications of these mutations.[110]

Table 1 | Genes involved in Brugada syndrome

Gene	Protein	Functional effect	OMIM[155] gene/locus number	OMIM[155] phenotype number
SCN5A	α Subunit cardiac Na^+ channel (Nav1.5)	Loss of function cardiac Na^+ current	600,163	601,144
GPD1L	Glycerol-3-phosphate dehydrogenase 1-like	Loss of function cardiac Na^+ current	611,778	611,777
CACNA1C	α Subunit cardiac Ca^{2+} channel ($Ca_v\alpha_{1c}$)	Loss of function cardiac L-type Ca^{2+} current (I_{CaL})	114,205	611,875
CACNB2	β_2 Subunit cardiac Ca^{2+} channel ($Ca_v\beta_{2b}$)	Loss of function cardiac L-type Ca^{2+} current (I_{CaL})	600,003	611,876
SCN1B	β_1 Subunit cardiac Na^+ channel	Loss of function cardiac Na^+ current	600,235	612,838
KCNE3	Accessory subunit of K^+ transient outward current (MiRP2)	Gain of function cardiac K^+ transient outward current	604,433	613,119
SCN3B	β_2 Subunit cardiac Na^+ channel	Loss of function cardiac Na^+ current	608,214	613,120
KCNJ8	α Subunit cardiac ATP K^+ channel ($K_{ir}6.1$)	Gain of function cardiac K^+-dependent current	600,935	NA
KCND3	$\alpha_2\delta$ Subunit cardiac transient outward K^+ channel ($K_v4.3$)	Gain of function cardiac K^+ transient outward current	605,411	NA
CACNA2D1	$\alpha_2\delta_1$ Subunit cardiac Ca^{2+} channel ($Ca_v\alpha2\delta1$)	Loss of function cardiac L-type Ca^{2+} current (I_{CaL})	114,204	NA
MOG1	Nucleocytoplasmic and microtubule transport	Loss of function cardiac Na^+ current	607,954	NA

Abbreviations: NA, not applicable; OMIM, Online Mendelian Inheritance in Man.

Loss-of-function mutations

Brugada syndrome

Brugada syndrome is characterized by ST-segment elevation with a 'coved'-type morphology in the right precordial leads, and is associated with an increased risk of sudden death secondary to polymorphic VT or VF.[111] Brugada syndrome has an estimated prevalence of 1 in 5,000 of the population.[112] In 10–30% of patients, Brugada syndrome is caused by mutations of *SCN5A*.[112] L-type Ca^{2+} channel mutations account for 10–15% of cases.[2] Mutations in other genes have also been reported (Table 1). Two hypotheses have been proposed to explain the link between ST-segment elevation and polymorphic VT in Brugada syndrome.[113] The repolarization hypothesis[114] proposes that the predominance of early depolarizing currents (transient outward current) over depolarizing currents (Na^+ and Ca^{2+} currents) causes loss of the action-potential dome and action-potential shortening in the epicardium of the right ventricular outflow tract. This process generates a transmural repolarization gradient that causes ST-segment elevation. Arrhythmias can be generated by this abnormal dispersion of action-potential duration, which causes the so-called phase 2 re-entry. The depolarization hypothesis suggests that the ST-segment elevation in the precordial leads is attributable to slowing of conduction and delayed activation of the right ventricular outflow tract.[115] This slowing of conduction provides the substrate for the generation of re-entry and arrhythmias.

Risk stratification is crucial in Brugada syndrome, because the only option for high-risk patients is the insertion of an ICD to prevent sudden death. The fundamentals of risk stratification in Brugada syndrome were identified a decade ago,[116] and have since been confirmed by several investigators.[117–119] More-recently, additional risk stratifiers have emerged,[117] among which the presence of QRS fragmentation seems the most promising.[117]

Although, to date, pharmacological therapy has not been proven to reduce the risk of VF, some agents have been reported to effectively prevent electrical storms or recurrent VF episodes. Infusion of the β-adrenergic agonist isoproterenol has been shown to suppress electrical storms,[120] whereas long-term treatment with the class I antiarrhythmic agent quinidine attenuates the ECG pattern observed in Brugada syndrome and seems to prevent recurrence of VF.[121,122]

Short QT syndrome

Short QT syndrome is a rare, genetic arrhythmia syndrome characterized by the combination of a short QT interval (the most-widely accepted cut-off is a corrected QT interval <350 ms)[123,124] and life-threatening ventricular arrhythmias. The prevalence of short QT interval is unknown, but is likely to be <1 in 10,000 of the

Table 2 | Genes involved in short QT syndrome

Gene	Protein	Functional effect	OMIM[155] gene/locus number	OMIM[155] phenotype number
KCNH2	α Subunit rapid component of delayed rectifier K^+ channel (HERG)	Gain of function rapid component of delayed rectifier K^+ current (I_{Kr})	152,427	609,620
KCNQ1	α Subunit slow component of delayed rectifier K^+ channel (K_vLQT1)	Gain of function slow component of delayed rectifier K^+ current (I_{Ks})	607,542	609,621
KCNJ2	α Subunit inward rectifier K^+ channel (K_{ir}2.1)	Gain of function of inward rectifier K^+ current (I_{K1})	600,681	609,622
CACNA1C	α Subunit cardiac L-type Ca^{2+} channel ($Ca_v\alpha_{1c}$)	Loss of function cardiac L-type Ca^{2+} current (I_{CaL})	114,205	NA
CACNB2	β_2 Subunit cardiac L-type Ca^{2+} channel ($Ca_v\beta_{2b}$)	Loss of function cardiac L-type Ca^{2+} current (I_{CaL})	600,003	NA
CACNA2D1	$\alpha_2\delta_1$ Subunit cardiac Ca^{2+} channel ($Ca_v\alpha_2\delta_1$)	Loss of function cardiac L-type Ca^{2+} current (I_{CaL})	114,204	NA

Abbreviations: NA, not applicable; OMIM, Online Mendelian Inheritance in Man.

Table 3 | Genes associated with idiopathic VF and early repolarization

Gene	Protein	Functional effect	OMIM[155] gene/locus number	OMIM[155] phenotype number
KCNJ8	α Subunit inward rectifier ATP sensitive K^+ channel (K_{ir}6.1)	Gain of function of inward rectifier ATP sensitive K^+ current (I_{KATP})	600,935	NA
CACNA1C	α Subunit cardiac L-type Ca^{2+} channel ($Ca_v\alpha_{1c}$)	Loss of function cardiac L-type Ca^{2+} current (I_{CaL})	114,205	NA
CACNB2	β_2 Subunit cardiac L-type Ca^{2+} channel ($Ca_v\beta_{2b}$)	Loss of function cardiac L-type Ca^{2+} current (I_{CaL})	600,003	NA
CACNA2D1	$\alpha_2\delta_1$ Subunit cardiac Ca^{2+} channel ($Ca_v\alpha_2\delta_1$)	Loss of function cardiac L-type Ca^{2+} current (I_{CaL})	114,204	NA
SCN5A	α Subunit cardiac Na^+ channel (Na_v1.5)	Loss of function cardiac Na^+ current	600,163	NA

Abbreviation: NA, not applicable; OMIM, Online Mendelian Inheritance in Man; VF, ventricular fibrillation.

population. Genetic analysis identifies mutations in around 20% of patients.[125]

Mutations of six different genes have been linked to short QT syndrome (Table 2). Three genes encoding cardiac K^+ currents and are affected by gain-of-function defects.[126–128] In the past 3 years, mutations of the genes encoding three L-type Ca^{2+} channel subunits have also been linked to short QT syndrome.[2,109] These mutations reduce I_{CaL} amplitude and shorten the action-potential duration and QT interval. Action-potential shortening might not be uniform across the ventricular wall, and can be more pronounced in the epicardium than in endocardium or midmyocardial cells.[129] This phenomenon causes an enhanced transmural dispersion of repolarization that facilitates the onset of re-entry and polymorphic VT.

Quinidine and sotalol have been reported to increase QT interval to a normal duration in some patients with short QT syndrome, and these drugs might also prevent VF.[130–132] Whether responsiveness to therapy with quinidine or sotalol varies with specific genetic variants of the disease remains unproven.

Early repolarization syndrome

Early repolarization pattern is defined as an elevation of the J-point associated with a small J-wave or slurring in the final part of the QRS complex.[133] Only in the past 3–5 years has this ECG pattern been reported to be associated with an increased risk of death. The diagnostic criteria have not been fully defined and, therefore, the prevalence of this condition is unknown. Among patients with idiopathic VF, this early repolarization pattern is highly prevalent in the inferior or inferolateral leads.[134,135] The incidence of sudden death caused by early repolarization in the general population is thought to be around 6 in 100,000 per year.[136] The highest level of risk seems to be associated with a >0.2 mV J-wave elevation,[137] with a flat or down-sloping ST segment.[138] Notably, even this latter pattern is associated with only a mild increase (up to 30 in 100,000 per year) in the incidence of sudden death.[139]

Mutations altering the functions of various channels have been linked with idiopathic VF related to early repolarization syndrome (Table 3). L-type Ca^{2+} channel mutations seem to account for 16% of cases.[2] The mechanism proposed to explain an early repolarization pattern in the inferior leads and the onset of arrhythmias is similar to the repolarization hypothesis proposed for Brugada syndrome.[140] The only difference is that early repolarization occurs at the level of the posterobasal epicardium instead of the right ventricular outflow tract.

Management strategies for patients with an early repolarization pattern on the ECG are still to be identified. Haïssaguerre *et al.* reported that, as for Brugada syndrome, isoproterenol infusion suppresses VT storms and long-term quinidine treatment prevents recurrence of arrhythmias in patients with early repolarization syndrome.[141] Currently, experts in the field generally agree that the presence of early repolarization in asymptomatic individuals is not to be considered a marker of electrical instability requiring medical attention, unless the electrocardiographic presentation is strikingly abnormal.

Overlapping syndromes

In the previous sections, we have outlined how mutations in the genes encoding the L-type Ca^{2+} channel can cause various clinical phenotypes. Interestingly, the coexistence of more than one of these clinical phenotypes in the same patient is also possible. Burashnikov *et al.* reported a short QT interval in five out of 25 patients with Brugada syndrome secondary to mutations in the genes encoding the L-type Ca^{2+} channel subunits.[2] Phenotypic overlap also exists between Brugada syndrome and early repolarization syndrome,[142] and between short QT syndrome and early repolarization syndrome.[143] Whether these overlaps are related to the specific functional effects of

mutations, or to the presence of additional modifiers such genetic polymorphisms, is unknown.

Gain-of-function mutations

Timothy syndrome

Timothy syndrome (TS) is a rare (<30 patients reported worldwide), multisystem disorder caused by mutations of *CACNA1C*, four of which have been described.[144,145] Two of these four mutations occur at the same position (amino acid 406) on exon 8 and exon 8a, which are alternatively spliced and produce the substitution of a glycine with an arginine (G406R).[146] The majority of patients with TS carry one of these mutations. TS is often fatal, and the only instances of familial transmission described so far are the result of parental mosaicism. In this situation, not all cell lines in the body carry the mutation.[1344,147] The mutation can be transmitted when present in the germinal line, but absent in the heart and central nervous system, thus explaining the lack of phenotype.

In vitro functional characterization of TS-causing mutant proteins has shown that voltage-dependent inactivation of the L-type Ca^{2+} channel is impaired, resulting in a persistent inward current that prolongs cardiac repolarization (that is, a gain of function).[148] In cardiac myocytes, a slowly inactivating Ca^{2+} current is produced, which causes prolongation of the action potential and the QT interval, as well as Ca^{2+} overload.[149] An important unresolved issue is the mechanistic connection between channel dysfunction and the extensive extracardiac manifestations of TS (discussed below), highlighting the ubiquitous expression of the channel. Pasca *et al.* generated neurons from inducible pluripotent stem cells derived from patients with TS, and demonstrated abnormalities in cell differentiation and increased production of norepinephrine and dopamine.[150] Bader and colleagues have constructed a knock-in mouse for the G406R mutation.[151] Interestingly, heterozygous mice developed only autistic behavior without any cardiac abnormalities.[151]

Phenotype and treatment of Timothy syndrome

Typically, the cardiac manifestations of TS include fetal bradycardia, severe prolongation of the QT interval (QT >500 ms) at birth with marked T wave alternans[144] (Figure 6), and 2:1 atrioventricular block at birth.[144] These abnormalities are often accompanied by congenital heart defects or cardiomyopathies. In addition, patients can develop polymorphic VT and VF within the first year of life. Extracardiac abnormalities include syndactyly, facial dysmorphism, autistic spectrum disorders, immunodeficiency, and severe hypoglycemia. Up to 80% of patients with TS die before puberty from cardiac arrhythmias (S. G. Priori, unpublished work). Deaths related to severe infection and intractable paroxysmal hypoglycemia have also been described (S. G. Priori, unpublished work).

The main focus of treatment in TS is prevention of life-threatening arrhythmias. Basic science studies and anecdotal reports suggest that Ca^{2+}-channel blockers,[152] β-blockers,[153] and the antianginal agent ranolazine[154] might have beneficial effects in these patients. In our experience, however, these drugs have a limited impact on the disease, and TS is fatal in most patients. Immunodeficiency caused by TS might increase the risk of ICD-related infection; therefore, ICDs should be used with caution in patients with TS. The prognosis is bleak in the majority of patients with TS, and the extent to which pharmacological or device therapies can modify the apparently adverse natural history of the disease is unclear. Experiments performed using inducible pluripotent stem cells derived from a patient with TS have indicated that roscovitine, a cyclin-dependent kinase inhibitor, normalizes Ca^{2+}-channel function and corrects most of the alterations caused by channel dysfunction.[99,100]

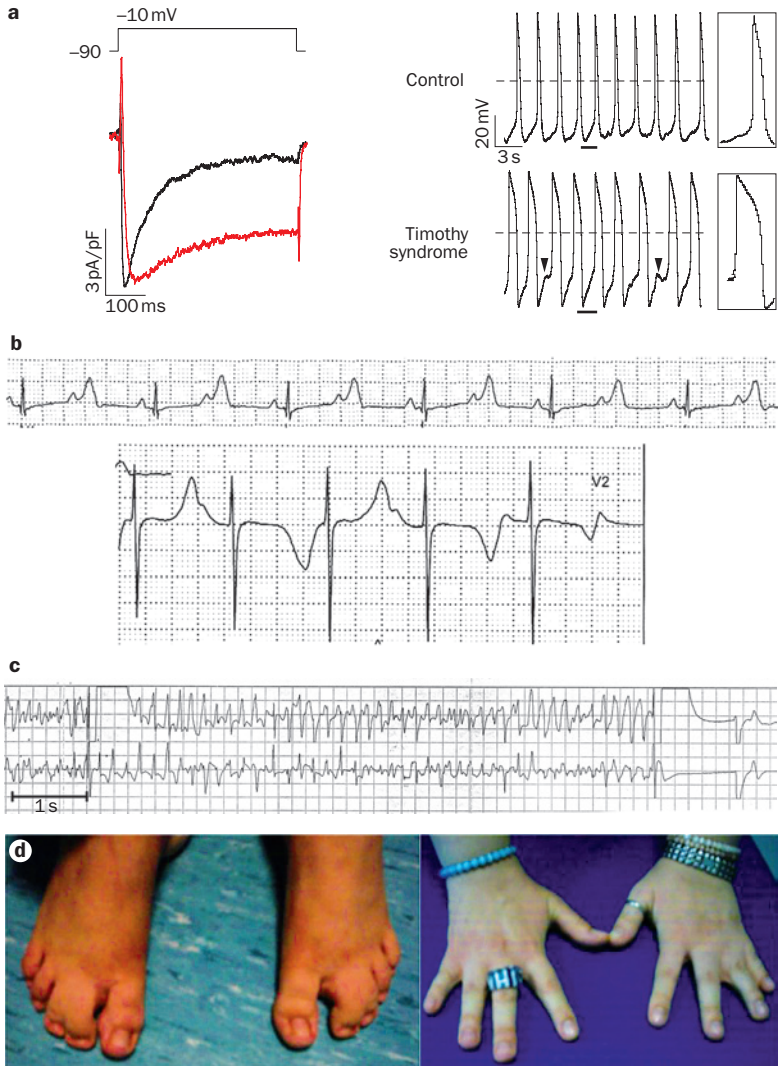

Figure 6 | Clinical manifestations of Timothy syndrome. **a** | Record of I_{CaL} (left) and action potentials (right) from inducible pluripotent stem cells derived from a patient with Timothy syndrome with the G406R mutation (red line) and a control individual (black line). The mutation slows the current inactivation and prolongs the action potential. **b** | Electrocardiogram from a patient with Timothy syndrome showing 2:1 atrioventricular block and macroscopic T wave alternans. **c** | Implantable cardioverter-defibrillator record of an episode of ventricular fibrillation in a patient with Timothy syndrome. **d** | Syndactyly in two patients with Timothy syndrome. Abbreviation: I_{CaL}, L-type calcium channel current. Permission for part a obtained from Nature Publishing Group © Yazawa, M. *et al. Nature* **471**, 230–234 (2011).

Thus, roscovitine could be a potential future therapy for patients with TS.

Conclusions

Advances in molecular genetics and cellular electrophysiology have uncovered genetic defects that alter calcium handling and lead to inherited arrhythmia syndromes. The cellular and subcellular pathophysiology of channelopathies caused by mutations in the *RYR2* gene and other genes that encode related proteins have been extensively studied and the fundamentals of the disease mechanisms, the clinical phenotypes, and potential treatment strategies have been characterized. Novel therapies designed on the basis of cellular and animal experimental models are being developed, although their clinical effectiveness still awaits confirmation in controlled clinical studies. Research should be fostered on additional factors, such as genetic polymorphisms, that modify phenotypic manifestations of channelopathies in the presence of a common genetic background. Furthermore, the fact that genetic mutations are identified in <100% of clinically affected individuals, suggests that not all causative genes have been identified. Some mutations might hit noncoding regions of the gene and affect channel structure or function by modifying the post-transcriptional processing of the gene transcripts.

An interesting issue is why mutations that reduce the I_{CaL} amplitude in inherited arrhythmogenic diseases do not affect cardiac contractility. Indeed, the negative inotropic effect of Ca^{2+}-channel blockers is well established. Therefore, the absence of reductions in cardiac contractility in patients with loss-of-function mutations is surprising. One possible explanation is that the reduction of Ca^{2+} current secondary to genetic mutations might not be severe enough to cause reduction in contractility. Alternatively, the existence of compensatory mechanisms could be hypothesized. From the clinical standpoint, with the exception of Brugada syndrome, the limited size of cohorts of patients with L-type Ca^{2+} channel mutations do not allow effective risk-stratification schemes to be delineated. Conceivably, over the next 2 decades, continuing research will enable us to move from disease-based treatments to gene-specific or mutation-specific treatments using both pharmacological and gene therapy.

Review criteria

We performed several searches of the PubMed database for original articles, reviews, and editorials. Search terms included "ryanodine receptor", "calsequestrin", "mutations", "arrhythmias", "Ca^{2+} overload", "L-type Ca^{2+} current", "Timothy syndrome", "Brugada syndrome", "short QT syndrome", and "early repolarization syndrome". These terms were used in various combinations. We selected only articles written in English. No date restrictions were applied to the search. We also checked the reference lists of the articles identified through these searches to identify further papers of interest.

1. Bers, D. M. Cardiac excitation–contraction coupling. *Nature* **415**, 198–205 (2002).
2. Burashnikov, E. *et al.* Mutations in the cardiac L-type calcium channel associated with inherited J-wave syndromes and sudden cardiac death. *Heart Rhythm* **7**, 1872–1882 (2010).
3. Priori, S. G. *et al.* Mutations in the cardiac ryanodine receptor gene (hRyR2) underlie catecholaminergic polymorphic ventricular tachycardia. *Circulation* **103**, 196–200 (2001).
4. Lahat, H. *et al.* A missense mutation in a highly conserved region of CASQ2 is associated with autosomal recessive catecholamine-induced polymorphic ventricular tachycardia in Bedouin families from Israel. *Am. J. Hum. Genet.* **69**, 1378–1384 (2001).
5. Hasenfuss, G. & Pieske, B. Calcium cycling in congestive heart failure. *J. Mol. Cell. Cardiol.* **34**, 951–969 (2002).
6. George, C. H. Sarcoplasmic reticulum Ca^{2+} leak in heart failure: mere observation or functional relevance? *Cardiovasc. Res.* **77**, 302–314 (2008).
7. Pogwizd, S. M. & Bers, D. M. Cellular basis of triggered arrhythmias in heart failure. *Trends Cardiovasc. Med.* **14**, 61–66 (2004).
8. Terentyev, D., Viatchenko-Karpinski, S., Valdivia, H. H., Escobar, A. L. & Györke, S. Luminal Ca^{2+} controls termination and refractory behavior of Ca^{2+}-induced Ca^{2+} release in cardiac myocytes. *Circ. Res.* **91**, 414–420 (2002).
9. Bassani J. W., Bassani, R. A. & Bers, D. M. Relaxation in rabbit and rat cardiac cells: species-dependent differences in cellular mechanisms. *J. Physiol.* **476**, 279–293 (1994).
10. Pieske, B., Maier, L. S., Bers, D. M. & Hassenfuss, G. Ca^{2+} handling and sarcoplasmic reticulum Ca^{2+} content in isolated failing and nonfailing human myocardium. *Circ. Res.* **85**, 38–46 (1999).
11. Cheng, H., Lederer, W. J. & Cannell, M. B. Calcium sparks: elementary events underlying excitation–contraction coupling in heart muscle. *Science* **262**, 740–744 (1993).
12. Franzini-Armstrong, C., Protasi, F. & Tijskens, P. The assembly of calcium release units in cardiac muscle. *Ann. NY Acad. Sci.* **1047**, 76–85 (2005).
13. Cannell, M. B., Cheng, H. & Lederer, W. J. The control of calcium release in heart muscle. *Science* **268**, 1045–1049 (1995).
14. Satoh, H., Blatter, L. A. & Bers, D. M. Effects of $[Ca^{2+}]_i$, SR Ca^{2+} load, and rest on Ca^{2+} spark frequency in ventricular myocytes. *Am. J. Physiol. Heart Circ. Physiol.* **272**, H657–H668 (1997).
15. Denegri, M. *et al.* Viral gene transfer rescues arrhythmogenic phenotype and ultrastructural abnormalities in adult calsequestrin-null mice with inherited arrhythmias. *Circ. Res.* **110**, 663–668 (2012).
16. Ginsburg, K. S. & Bers, D. M. Modulation of excitation–contraction coupling by isoproterenol in cardiomyocytes with controlled SR Ca^{2+} load and Ca^{2+} current trigger. *J. Physiol.* **556**, 463–480 (2004).
17. Hussain, M. & Orchard, C. H. Sarcoplasmic reticulum Ca^{2+} content, L-type Ca^{2+} current and the Ca^{2+} transient in rat myocytes during beta-adrenergic stimulation. *J. Physiol.* **505**, 385–402 (1997).
18. Grimm, M. & Brown, J. H. Beta-adrenergic receptor signaling in the heart: role of CaMKII. *J. Mol. Cell. Cardiol.* **48**, 322–330 (2010).
19. Reiken, S. *et al.* Protein kinase A phosphorylation of the cardiac calcium release channel (ryanodine receptor) in normal and failing hearts. Role of phosphatases and response to isoproterenol. *J. Biol. Chem.* **278**, 444–453 (2003).
20. Wehrens, X. H., Lehnart, S. E., Reiken, S. R. & Marks, A. R. Ca^{2+}/calmodulin-dependent protein kinase II phosphorylation regulates the cardiac ryanodine receptor. *Circ. Res.* **94**, e61–e70 (2004).
21. MacDonnell, S. M. *et al.* Adrenergic regulation of cardiac contractility does not involve phosphorylation of the cardiac ryanodine receptor at serine 2808. *Circ. Res.* **102**, e65–e72 (2008).
22. Marx, S. O. *et al.* PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. *Cell* **101**, 365–376 (2000).
23. Ai, X., Curran, J. W., Shannon, T. R., Bers, D. M. & Pogwizd S. M. Ca^{2+}/calmodulin-dependent protein kinase modulates cardiac ryanodine receptor phosphorylation and sarcoplasmic reticulum Ca^{2+} leak in heart failure. *Circ. Res.* **97**, 1314–1322 (2005).
24. Bers, D. M. Ryanodine receptor S2808 phosphorylation in heart failure: smoking gun or red herring. *Circ. Res.* **110**, 796–799 (2012).
25. Maier, L. S. & Bers, D. M. Role of Ca^{2+}/calmodulin-dependent protein kinase (CaMK) in excitation–contraction coupling in the heart. *Cardiovasc. Res.* **73**, 631–640 (2007).
26. Priori, S. G. *et al.* Clinical and molecular characterization of patients with catecholaminergic polymorphic ventricular tachycardia. *Circulation* **106**, 69–74 (2002).
27. Laitinen, P. J. *et al.* Mutations of the cardiac ryanodine receptor (*RyR2*) gene in familial polymorphic ventricular tachycardia. *Circulation* **103**, 485–490 (2001).

28. Bhuiyan, Z. A. *et al.* Expanding spectrum of human RYR2-related disease: new electrocardiographic, structural, and genetic features. *Circulation* **116**, 1569–1576 (2007).
29. Tang, Y., Tian, X., Wang, R., Fill, M. & Chen, S. R. Abnormal termination of Ca^{2+} release is a common defect of *RyR2* mutations associated with cardiomyopathies. *Circ. Res.* **110**, 968–977 (2012).
30. Tiso, N. *et al.* Identification of mutations in the cardiac ryanodine receptor gene in families affected with arrhythmogenic right ventricular cardiomyopathy type 2 (ARVD2). *Hum. Mol. Genet.* **10**, 189–194 (2001).
31. Venetucci, L. A., Trafford, A. W., O'Neill, S. C. & Eisner, D. A. The sarcoplasmic reticulum and arrhythmogenic calcium release. *Cardiovasc. Res.* **77**, 285–292 (2008).
32. Cheng, H., Lederer, M. R., Lederer, W. J. & Cannell, M. B. Calcium sparks and $[Ca^{2+}]_i$ waves in cardiac myocytes. *Am. J. Physiol.* **270**, C148–C159 (1996).
33. Lukyanenko, V., Subramanian, S., Györke, I., Wiesner, T. F. & Györke, S. The role of luminal Ca^{2+} in the generation of Ca^{2+} waves in rat ventricular myocytes. *J. Physiol.* **518**, 173–186 (1999).
34. Venetucci, L. A., Trafford, A. W. & Eisner, D. A. Increasing ryanodine receptor open probability alone does not produce arrhythmogenic calcium waves: threshold sarcoplasmic reticulum calcium content is required. *Circ. Res.* **100**, 105–111 (2007).
35. Trafford, A. W., Sibbring, G. C., Díaz, M. E. & Eisner, D. A. The effects of low concentrations of caffeine on spontaneous Ca release in isolated rat ventricular myocytes. *Cell Calcium* **28**, 269–276 (2000).
36. Overend, C. L., Eisner, D. A. & O'Neill, S. C. The effect of tetracaine on spontaneous Ca^{2+} release and sarcoplasmic reticulum calcium content in rat ventricular myocytes. *J. Physiol.* **502**, 471–479 (1997).
37. Lederer, W. J. & Tsien, R. W. Transient inward current underlying arrhythmogenic effects of cardiotonic steroids in Purkinje fibers. *J. Physiol.* **263**, 73–100 (1976).
38. Jiang, D. *et al.* Enhanced store overload-induced Ca^{2+} release and channel sensitivity to luminal Ca^{2+} activation are common defects of *RyR2* mutations linked to ventricular tachycardia and sudden death. *Circ. Res.* **97**, 1173–1181 (2005).
39. Jiang, D. *et al. RyR2* mutations linked to ventricular tachycardia and sudden death reduce the threshold for store-overload-induced Ca^{2+} release (SOICR). *Proc. Natl Acad. Sci. USA* **101**, 13062–13067 (2004).
40. Terentyev, D. *et al.* Abnormal interactions of calsequestrin with the ryanodine receptor calcium release channel complex linked to exercise-induced sudden cardiac death. *Circ. Res.* **98**, 1151–1158 (2006).
41. Liu, N. *et al.* Arrhythmogenesis in catecholaminergic polymorphic ventricular tachycardia: insights from a *RyR2* R4496C knock-in mouse model. *Circ. Res.* **99**, 292–298 (2006).
42. George, C. H., Jundi, H., Thomas, N. L., Fry, D. L. & Lai, F. A. Ryanodine receptors and ventricular arrhythmias: emerging trends in mutations, mechanisms and therapies. *J. Mol. Cell. Cardiol.* **42**, 34–50 (2007).
43. Medeiros-Domingo, A. *et al.* The *RYR2*-encoded ryanodine receptor/calcium release channel in patients diagnosed previously with either catecholaminergic polymorphic ventricular tachycardia or genotype negative, exercise-induced long QT syndrome: a comprehensive open reading frame mutational analysis. *J. Am. Coll. Cardiol.* **54**, 2065–2074 (2009).
44. Rousseau, E., Smith, J. S., Henderson, J. S. & Meissner, G. Single channel and $^{45}Ca^{2+}$ flux measurements of the cardiac sarcoplasmic reticulum calcium channel. *Biophys. J.* **50**, 1009–1014 (1986).
45. Sitsapesan, R. & Williams, A. J. Regulation of the gating of the sheep cardiac sarcoplasmic reticulum Ca^{2+}-release channel by luminal Ca^{2+}. *J. Membr. Biol.* **137**, 215–226 (1994).
46. Stern, M. D. & Cheng, H. Putting out the fire: what terminates calcium-induced calcium release in cardiac muscle? *Cell Calcium* **35**, 591–601 (2004).
47. IRCCS Fondazione Salvatore Maugeri, Italy and New York University, USA. *The gene connection for the heart. Genetic mutations and inherited arrhythmias* [online], www.fsm.it/cardmoc (2010).
48. Jones, P. P. *et al.* Endoplasmic reticulum Ca^{2+} measurements reveal that the cardiac ryanodine receptor mutations linked to cardiac arrhythmia and sudden death alter the threshold for store-overload-induced Ca^{2+} release. *Biochem. J.* **412**, 171–178 (2008).
49. Lehnart, S. E. *et al.* Sudden death in familial polymorphic ventricular tachycardia associated with calcium release channel (ryanodine receptor) leak. *Circulation* **109**, 3208–3214 (2004).
50. Wehrens, X. H. *et al.* FKBP12.6 deficiency and defective calcium release channel (ryanodine receptor) function linked to exercise-induced sudden cardiac death. *Cell* **113**, 829–840 (2003).
51. George, C. H., Higgs, G. V. & Lai, F. A. Ryanodine receptor mutations associated with stress-induced ventricular tachycardia mediate increased calcium release in stimulated cardiomyocytes. *Circ. Res.* **93**, 531–540 (2003).
52. Guo, T. *et al.* Kinetics of FKBP12.6 binding to ryanodine receptors in permeabilized cardiac myocytes and effects on Ca sparks. *Circ. Res.* **106**, 1743–1752 (2010).
53. Kashimura, T. *et al.* In the RyR2(R4496C) mouse model of CPVT, β-adrenergic stimulation induces Ca waves by increasing SR Ca content and not by decreasing the threshold for Ca waves. *Circ. Res.* **107**, 1483–1489 (2010).
54. Yamamoto, T. *et al.* Identification of target domains of the cardiac ryanodine receptor to correct channel disorder in failing hearts. *Circulation* **117**, 762–772 (2008).
55. Uchinoumi, H. *et al.* Catecholaminergic polymorphic ventricular tachycardia is caused by mutation-linked defective conformational regulation of the ryanodine receptor. *Circ. Res.* **106**, 1413–1424 (2010).
56. Suetomi, T. *et al.* Mutation-linked defective interdomain interactions within ryanodine receptor cause aberrant Ca^{2+} release leading to catecholaminergic polymorphic ventricular tachycardia. *Circulation* **124**, 682–694 (2011).
57. Jiang, D., Chen, W., Wang, R., Zhang, L. & Chen, S. R. Loss of luminal Ca^{2+} activation in the cardiac ryanodine receptor is associated with ventricular fibrillation and sudden death. *Proc. Natl Acad. Sci. USA* **104**, 18309–18314 (2007).
58. Györke, S. & Terentyev, D. Modulation of ryanodine receptor by luminal calcium and accessory proteins in health and cardiac disease. *Cardiovasc. Res.* **77**, 245–255 (2008).
59. Wang, S. *et al.* Crystal structure of calsequestrin from rabbit skeletal muscle sarcoplasmic reticulum. *Nat. Struct. Biol.* **5**, 476–483 (1998).
60. Mitchell, R. D., Simmerman, H. K. & Jones, L. R. Ca^{2+} binding effects on protein conformation and protein interactions of canine cardiac calsequestrin. *J. Biol. Chem.* **263**, 1376–1381 (1988).
61. Györke, I., Hester, N., Jones, L. R. & Györke, S. The role of calsequestrin, triadin, and junctin in conferring cardiac ryanodine receptor responsiveness to luminal calcium. *Biophys. J.* **86**, 2121–2128 (2004).
62. Xu, L. & Meissner, G. Regulation of cardiac muscle Ca^{2+} release channel by sarcoplasmic reticulum lumenal Ca^{2+}. *Biophys. J.* **75**, 2302–2312 (1998).
63. Knollmann, B. C. *et al. Casq2* deletion causes sarcoplasmic reticulum volume increase, premature Ca^{2+} release, and catecholaminergic polymorphic ventricular tachycardia. *J. Clin. Invest.* **116**, 2510–2520 (2006).
64. Qin, J. *et al.* Luminal Ca^{2+} regulation of single cardiac ryanodine receptors: insights provided by calsequestrin and its mutants. *J. Gen. Physiol.* **131**, 325–334 (2008).
65. di Barletta, M. R. *et al.* Clinical phenotype and functional characterization of *CASQ2* mutations associated with catecholaminergic polymorphic ventricular tachycardia. *Circulation* **114**, 1012–1019 (2006).
66. de la Fuente, S., Van Langen, I. M., Postma, A. V., Bikker, H. & Meijer, A. A case of catecholaminergic polymorphic ventricular tachycardia caused by two calsequestrin 2 mutations. *Pacing Clin. Electrophysiol.* **31**, 916–919 (2008).
67. Rizzi, N. *et al.* Unexpected structural and functional consequences of the R33Q homozygous mutation in cardiac calsequestrin: a complex arrhythmogenic cascade in a knock-in mouse model. *Circ. Res.* **103**, 298–306 (2008).
68. Song, L. *et al.* Calsequestrin 2 (*CASQ2*) mutations increase expression of calreticulin and ryanodine receptors, causing catecholaminergic polymorphic ventricular tachycardia. *J. Clin. Invest.* **117**, 1814–1823 (2007).
69. Kalyanasundaram, A., Bal, N. C., Franzini-Armstrong, C., Knollmann, B. C. & Periasamy, M. The calsequestrin mutation CASQ2D307H does not affect protein stability and targeting to the junctional sarcoplasmic reticulum but compromises its dynamic regulation of calcium buffering. *J. Biol. Chem.* **285**, 3076–3083 (2010).
70. Bal, N. C. *et al.* The catecholaminergic polymorphic ventricular tachycardia mutation R33Q disrupts the N-terminal structural motif that regulates reversible calsequestrin polymerization. *J. Biol. Chem.* **285**, 17188–17196 (2010).
71. Valle, G. *et al.* Catecholaminergic polymorphic ventricular tachycardia-related mutations R33Q and L167H alter calcium sensitivity of human cardiac calsequestrin. *Biochem. J.* **413**, 291–303 (2008).
72. Houle, T. D., Ram, M. L. & Cala, S. E. Calsequestrin mutant D307H exhibits depressed binding to its protein targets and a depressed response to calcium. *Cardiovasc. Res.* **64**, 227–233 (2004).
73. Eisner, D. A., Trafford, A. W., Díaz, M. E., Overend, C. L. & O'Neill, S. C. The control of Ca release from the cardiac sarcoplasmic reticulum: regulation versus autoregulation. *Cardiovasc. Res.* **38**, 589–604 (1998).
74. Liu, N. *et al.* Calmodulin kinase II inhibition prevents arrhythmias in *RyR2*(R4496C±) mice with catecholaminergic polymorphic ventricular tachycardia. *J. Mol. Cell. Cardiol.* **50**, 214–222 (2011).

75. Curran, J., Brown, K. H., Santiago, D. J., Pogwizd, S., Bers, D. M. & Shannon, T. R. Spontaneous Ca waves in ventricular myocytes from failing hearts depend on Ca^{2+}-calmodulin-dependent protein kinase II. *J. Mol. Cell. Cardiol.* **49**, 25–32 (2010).
76. Leenhardt, A. *et al.* Catecholaminergic polymorphic ventricular tachycardia in children. A 7-year follow-up of 21 patients. *Circulation* **91**, 1512–1519 (1995).
77. Postma, A. V. *et al.* Catecholaminergic polymorphic ventricular tachycardia: *RYR2* mutations, bradycardia, and follow up of the patients. *J. Med. Genet.* **42**, 863–870 (2005).
78. Marjamaa, A. *et al.* Intravenous epinephrine infusion test in diagnosis of catecholaminergic polymorphic ventricular tachycardia. *J. Cardiovasc. Electrophysiol.* **23**, 194–199 (2011).
79. Landzberg, S., Parker, J. D., Gauthier, D. F. & Colucci, W. S. Effects of intracoronary acetylcholine and atropine on basal and dobutamine-stimulated left ventricular contractility. *Circulation* **89**, 164–168 (1994).
80. Mohamed, U., Gollob, M. H., Gow, R. M. & Krahn, A. D. Sudden cardiac death despite an implantable cardioverter-defibrillator in a young female with catecholaminergic ventricular tachycardia. *Heart Rhythm* **3**, 1486–1489 (2006).
81. Odero, A., Bozzani, A., De Ferrari, G. M. & Schwartz, P. J. Left cardiac sympathetic denervation for the prevention of life-threatening arrhythmias: the surgical supraclavicular approach to cervicothoracic sympathectomy. *Heart Rhythm* **7**, 1161–1165 (2010).
82. Collura, C. A., Johnson, J. N., Moir, C. & Ackerman, M. J. Left cardiac sympathetic denervation for the treatment of long QT syndrome and catecholaminergic polymorphic ventricular tachycardia using video-assisted thoracic surgery. *Heart Rhythm* **6**, 752–759 (2009).
83. Wilde, A. A. *et al.* Left cardiac sympathetic denervation for catecholaminergic polymorphic ventricular tachycardia. *N. Engl. J. Med.* **358**, 2024–2029 (2008).
84. Watanabe, H. *et al.* Flecainide prevents catecholaminergic polymorphic ventricular tachycardia in mice and humans. *Nat. Med.* **15**, 380–383 (2009).
85. van der Werf, C. *et al.* Flecainide therapy reduces exercise-induced ventricular arrhythmias in patients with catecholaminergic polymorphic ventricular tachycardia. *J. Am. Coll. Cardiol.* **57**, 2244–2254 (2011).
86. Hilliard, F. A. *et al.* Flecainide inhibits arrhythmogenic Ca^{2+} waves by open state block of ryanodine receptor Ca^{2+} release channels and reduction of Ca^{2+} spark mass. *J. Mol. Cell. Cardiol.* **48**, 293–301 (2010).
87. Conard, G. J. & Ober, R. E. Metabolism of flecainide. *Am. J. Cardiol.* **53**, 41B–51B (1984).
88. Liu, H., Atkins, J. & Kass, R. S. Common molecular determinants of flecainide and lidocaine block of heart Na^+ channels: evidence from experiments with neutral and quaternary flecainide analogs. *J. Gen. Physiol.* **121**, 199–214 (2003).
89. Liu, N. *et al.* Short communication: flecainide exerts an antiarrhythmic effect in a mouse model of catecholaminergic polymorphic ventricular tachycardia by increasing the threshold for triggered activity. *Circ. Res.* **109**, 291–295 (2011).
90. Lehnart, S. E. *et al.* Sudden death in familial polymorphic ventricular tachycardia associated with calcium release channel (ryanodine receptor) leak. *Circulation* **109**, 3208–3214 (2004).
91. Loughrey, C. M. *et al.* K201 modulates excitation–contraction coupling and spontaneous Ca^{2+} release in normal adult rabbit ventricular cardiomyocytes. *Cardiovasc. Res.* **76**, 236–246 (2007).
92. Sedej, S. *et al.* Na^+-dependent SR Ca^{2+} overload induces arrhythmogenic events in mouse cardiomyocytes with a human CPVT mutation. *Cardiovasc. Res.* **87**, 50–59 (2010).
93. Napolitano, C. & Antzelevitch, C. Phenotypical manifestations of mutations in the genes encoding subunits of the cardiac voltage-dependent L-type calcium channel. *Circ. Res.* **108**, 607–618 (2011).
94. Yuan, W., Ginsburg, K. S. & Bers, D. M. Comparison of sarcolemmal calcium channel current in rabbit and rat ventricular myocytes. *J. Physiol.* **493**, 733–746 (1996).
95. Bers, D. M. & Perez-Reyes, E. Ca channels in cardiac myocytes: structure and function in Ca influx and intracellular Ca release. *Cardiovasc. Res.* **42**, 339–360 (1999).
96. Zhang, Z. *et al.* Functional roles of $Ca_v1.3(\alpha_{1D})$ calcium channels in sinoatrial nodes: insights gained from gene-targeted null mutant mice. *Circ. Res.* **90**, 981–987 (2002).
97. Mikami, A. *et al.* Primary structure and functional expression of the cardiac dihydropyridine-sensitive calcium channel. *Nature* **340**, 230–233 (1989).
98. Hulme, J. T., Yarov-Yarovoy, V., Lin, T. W., Scheuer, T. & Catterall, W. A. Autoinhibitory control of the CaV1.2 channel by its proteolytically processed distal C-terminal domain. *J. Physiol.* **576**, 87–102 (2006).
99. Fu, Y. *et al.* Deletion of the distal C terminus of CaV1.2 channels leads to loss of beta-adrenergic regulation and heart failure *in vivo*. *J. Biol. Chem.* **286**, 12617–12626 (2011).
100. Catterall, W. A. Voltage-gated calcium channels. *Cold Spring Harb. Perspect. Biol.* **3**, a003947 (2011).
101. Davies, A. *et al.* The α2δ subunits of voltage-gated calcium channels form GPI-anchored proteins, a post-translational modification essential for function. *Proc. Natl Acad. Sci. USA* **107**, 1654–1659 (2010).
102. Fuller-Bicer, G. A. *et al.* Targeted disruption of the voltage-dependent calcium channel α_2/δ-1-subunit. *Am. J. Physiol. Heart Circ. Physiol.* **297**, H117–H124 (2009).
103. Colecraft, H. M. *et al.* Novel functional properties of Ca^{2+} channel beta subunits revealed by their expression in adult rat heart cells. *J. Physiol.* **541**, 435–452 (2002).
104. Buraei, Z. & Yang, J. The β subunit of voltage-gated Ca^{2+} channels. *Physiol. Rev.* **90**, 1461–1506 (2010).
105. Ruan, Y., Liu, N. & Priori, S. G. Sodium channel mutations and arrhythmias. *Nat. Rev. Cardiol.* **6**, 337–348 (2009).
106. Bezzina, C. *et al.* A single Na^+ channel mutation causing both long-QT and Brugada syndromes. *Circ. Res.* **85**, 1206–1213 (1999).
107. Antzelevitch, C. *et al.* Loss-of-function mutations in the cardiac calcium channel underlie a new clinical entity characterized by ST-segment elevation, short QT intervals, and sudden cardiac death. *Circulation* **115**, 442–449 (2007).
108. Cordeiro, J. M. *et al.* Accelerated inactivation of the L-type calcium current due to a mutation in *CACNB2b* underlies Brugada syndrome. *J. Mol. Cell. Cardiol.* **46**, 695–703 (2009).
109. Templin, C. *et al.* Identification of a novel loss-of-function calcium channel gene mutation in short QT syndrome (SQTS6). *Eur. Heart J.* **32**, 1077–1088 (2011).
110. Gollob, M. H. *et al.* Recommendations for the use of genetic testing in the clinical evaluation of inherited cardiac arrhythmias associated with sudden cardiac death: Canadian Cardiovascular Society/Canadian Heart Rhythm Society joint position paper. *Can. J. Cardiol.* **27**, 232–245 (2011).
111. Brugada, P. & Brugada, J. Right bundle branch block, persistent ST segment elevation and sudden cardiac death: a distinct clinical and electrocardiographic syndrome. A multicenter report. *J. Am. Coll. Cardiol.* **20**, 1391–1396 (1992).
112. Antzelevitch, C. *et al.* Brugada syndrome: report of the second consensus conference: endorsed by the Heart Rhythm Society and the European Heart Rhythm Association. *Circulation* **111**, 659–670 (2005).
113. Wilde, A. A. *et al.* The pathophysiological mechanism underlying Brugada syndrome: depolarization versus repolarization. *J. Mol. Cell. Cardiol.* **49**, 543–553 (2010).
114. Yan, G. X. & Antzelevitch, C. Cellular basis for the Brugada syndrome and other mechanisms of arrhythmogenesis associated with ST-segment elevation. *Circulation* **100**, 1660–1666 (1999).
115. Meregalli, P. G., Wilde, A. A. & Tan, H. L. Pathophysiological mechanisms of Brugada syndrome: depolarization disorder, repolarization disorder, or more? *Cardiovasc. Res.* **67**, 367–378 (2005).
116. Priori, S. G. *et al.* Natural history of Brugada syndrome: insights for risk stratification and management. *Circulation* **105**, 1342–1347 (2002).
117. Priori, S. G. *et al.* Risk stratification in Brugada syndrome: results of the PRELUDE (PRogrammed ELectrical stimUlation preDictive valuE) registry. *J. Am. Coll. Cardiol.* **59**, 37–45 (2012).
118. Probst, V. *et al.* Long-term prognosis of patients diagnosed with Brugada syndrome: results from the FINGER Brugada Syndrome Registry. *Circulation* **121**, 635–643 (2010).
119. Gehi, A. K., Duong, T. D., Metz, L. D., Gomes, J. A. & Mehta, D. Risk stratification of individuals with the Brugada electrocardiogram: a meta-analysis. *J. Cardiovasc. Electrophysiol.* **17**, 577–583 (2006).
120. Ohgo, T. *et al.* Acute and chronic management in patients with Brugada syndrome associated with electrical storm of ventricular fibrillation. *Heart Rhythm* **4**, 695–700 (2007).
121. Belthassen, B., Glick, A. & Viskin, S. Efficacy of quinidine in high-risk patients with Brugada syndrome. *Circulation* **110**, 1731–1737 (2004).
122. Antzelevitch, C. & Fish, J. M. Therapy for the Brugada syndrome. *Handb. Exp. Pharmacol.* **171**, 305–330 (2006).
123. Bjerregaard, P., Nallapaneni, H. & Gussak, I. Short QT interval in clinical practice. *J. Electrocardiol.* **43**, 390–395 (2010).
124. Viskin, S. The QT interval: too long, too short or just right. *Heart Rhythm* **6**, 711–715 (2009).
125. Gollob, M. H., Redpath, C. J. & Roberts, J. D. The short QT syndrome: proposed diagnostic criteria. *J. Am. Coll. Cardiol.* **57**, 802–812 (2011).
126. Brugada, R. *et al.* Sudden death associated with short-QT syndrome linked to mutations in *HERG*. *Circulation* **109**, 30–35 (2004).
127. Bellocq, C. *et al.* Mutation in the *KCNQ1* gene leading to the short QT-interval syndrome. *Circulation* **109**, 2394–2397 (2004).
128. Priori, S. G. *et al.* A novel form of short QT syndrome (SQT3) is caused by a mutation in the *KCNJ2* gene. *Circ. Res.* **96**, 800–807 (2005).
129. Patel, C., Yan, G. X. & Antzelevitch, C. Short QT syndrome: from bench to bedside. *Circ. Arrhythm. Electrophysiol.* **3**, 401–408 (2010).

130. Gaita, F. *et al.* Short QT syndrome: pharmacologic treatment. *J. Am. Coll. Cardiol.* **43**, 1494–1499 (2004).
131. Giustetto, C. *et al.* Long term follow-up of patients with short QT syndrome. *J. Am. Coll. Cardiol.* **58**, 587–595 (2011).
132. Iost, N., Viràg, L., Varrò, A. & Papp, J. G. Comparison of the effect of class IA antiarrhythmic drugs on transmembrane potassium currents in rabbit ventricular myocytes. *J. Cardiovasc. Pharmacol. Ther.* **8**, 31–41 (2003).
133. Boineau, J. P. The early repolarization variant —normal or a marker of heart disease in certain subjects. *J. Electrocardiol.* **40**, 3.e11–3.e16 (2007).
134. Haïssaguerre, M. *et al.* Sudden cardiac arrest associated with early repolarization. *N. Engl. J. Med.* **358**, 2016–2023 (2008).
135. Rosso, R. *et al.* J-point elevation in survivors of primary ventricular fibrillation and matched control subjects: incidence and clinical significance. *J. Am Coll. Cardiol.* **52**, 1231–1238 (2008).
136. Zipes, D. P. *et al.* ACC/AHA/ESC 2006 guidelines for management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: a report of the American College of Cardiology/American Heart Association Task Force and the European Society of Cardiology Committee for Practice Guidelines (Writing Committee to Develop Guidelines for Management of Patients With Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death). *J. Am. Coll. Cardiol.* **48**, e247–e346 (2006).
137. Rosso, R., Adler, A., Halkin, A. & Viskin, S. Risk of sudden death among young individuals with J waves and early repolarization: putting the evidence into perspective. *Heart Rhythm* **8**, 923–929 (2011).
138. Tikkanen, J. T. *et al.* Early repolarization: electrocardiographic phenotypes associated with favorable long-term outcome. *Circulation* **123**, 2666–2673 (2011).
139. Rosso, R. *et al.* Distinguishing "benign" from "malignant early repolarization": the value of the ST-segment morphology. *Heart Rhythm* **9**, 225–229 (2011).
140. Antzelevitch, C. & Yan, G. X. J-wave syndromes: from cell to bedside. *J. Electrocardiol.* **44**, 656–661 (2011).
141. Haïssaguerre, M. *et al.* Characteristics of recurrent ventricular fibrillation associated with inferolateral early repolarization role of drug therapy. *J. Am. Coll. Cardiol.* **53**, 612–619 (2009).
142. Letsas, K. P. *et al.* Prevalence of early repolarization pattern in inferolateral leads in patients with Brugada syndrome. *Heart Rhythm* **5**, 1685–1689 (2008).
143. Watanabe, H. *et al.* High prevalence of early repolarization in short QT syndrome. *Heart Rhythm* **7**, 647–652 (2010).
144. Splawski, I. *et al.* Ca(V)1.2 calcium channel dysfunction causes a multisystem disorder including arrhythmia and autism. *Cell* **119**, 19–31 (2004).
145. Gillis, J. *et al.* Long QT, syndactyly, joint contractures, stroke and novel *CACNA1C* mutation: expanding the spectrum of Timothy syndrome. *Am. J. Med. Genet. A.* http://dx.doi.org/10.1002/ajmg.a.34355.
146. Splawski, I. *et al.* Severe arrhythmia disorder caused by cardiac L-type calcium channel mutations. *Proc. Natl Acad. Sci. USA* **102**, 8089–8096 (2005).
147. Etheridge, S. P. *et al.* Somatic mosaicism contributes to phenotypic variation in Timothy syndrome. *Am. Med. Genet. A.* **155**, 2578–2583 (2011).
148. Barrett, C. F. & Tsien, R. W. The Timothy syndrome mutation differentially affects voltage- and calcium-dependent inactivation of CaV1.2 L-type calcium channels. *Proc. Natl Acad. Sci. USA* **105**, 2157–2162 (2008).
149. Yazawa, M. *et al.* Using induced pluripotent stem cells to investigate cardiac phenotypes in Timothy syndrome. *Nature* **471**, 230–234 (2011).
150. Paşca, S. P. *et al.* Using iPSC-derived neurons to uncover cellular phenotypes associated with Timothy syndrome. *Nat. Med.* **17**, 1657–1662 (2011).
151. Bader, P. L. *et al.* Mouse model of Timothy syndrome recapitulates triad of autistic traits. *Proc. Natl Acad. Sci. USA* **108**, 15432–15437 (2011).
152. Jacobs, A., Knight, B. P., McDonald, K. T. & Burke, M. C. Verapamil decreases ventricular tachyarrhythmias in a patient with Timothy syndrome (LQT8). *Heart Rhythm* **3**, 967–970 (2006).
153. Sung, R. J. *et al.* Beta-adrenergic modulation of arrhythmogenesis and identification of targeted sites of antiarrhythmic therapy in Timothy (LQT8) syndrome: a theoretical study. *Am. J. Physiol. Heart Circ. Physiol.* **298**, H33–H44 (2010).
154. Sicouri, S. *et al.* Cellular basis for the electrocardiographic and arrhythmic manifestations of Timothy syndrome: effects of ranolazine. *Heart Rhythm* **4**, 638–647 (2007).
155. Johns Hopkins University and US National Library of Medicine. *Online Mendelian Inheritance in Man (OMIM)* [online], http://www.ncbi.nlm.nih.gov/omim (2012).

Acknowledgments
Part of the data presented here has been produced thanks to the contribution of the following research grants: Telethon grants GGP04066, GGP06007, and GGP11141 (to S. G. Priori), Italian University Research and Technology RFMAU207641137D (to S. G. Priori), Fondation Leducq Award to the Alliance for Calmodulin Kinase Signaling in Heart Disease (08CVD01) (to S. G. Priori), Fondation Veronesi "Modelli cellulari e terapia sperimentale dei difetti cardiaci associati a patologie aritmogene ereditarie" (to S. G. Priori). CARIPLO pr.2008.2275 (to S. G. Priori), and British Heart Foundation Intermediate Clinical Research Fellowship FS10/63/28,374 (to L. Venetucci and S. G. Priori).

Author contributions
All authors contributed to the discussion of content for this Review. In addition, L. Venetucci researched data for and wrote the article, M. Denegri and C. Napolitano reviewed/edited the manuscript before submission, and S. G. Priori wrote the article and reviewed/edited the manuscript before submission.