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Calsequestrin Mutations and Catecholaminergic Polymorphic Ventricular Tachycardia

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Abstract

Cardiac calsequestrin (Casq2) is the major Ca^{2+} binding protein in the sarcoplasmic reticulum which is the principle Ca^{2+} storage organelle of cardiac muscle. Over the last decade, experimental studies have provided new concepts on the role of Casq2 in the regulation of cardiac muscle Ca^{2+} handling. Furthermore, mutations in the gene encoding for cardiac calsequestrin, *CASQ2*, cause a rare but severe form of Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT). Here, we review the physiology of Casq2 in cardiac Ca^{2+} handling and discuss pathophysiological mechanisms that lead to CPVT caused by *CASQ2* mutations. We also describe the clinical aspects of CPVT and provide an update of its contemporary clinical management.

1 Introduction

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a genetic arrhythmic disorder characterized by ventricular arrhythmias, usually provoked by emotional stress or exercise, in the absence of any structural cardiac abnormality. It usually presents in young individuals in the first or second decade of life and is regarded as a highly lethal disease with an overall mortality rate of 30–40% if left untreated [54]. On a molecular level, CPVT is caused by impaired intracellular Ca^{2+} handling due to mutations in genes encoding for the cardiac ryanodine receptor (RyR2) [64, 73] and cardiac calsequestrin (Casq2) [43], two main proteins forming the Ca^{2+} release unit of the sarcoplasmic reticulum (SR). *RYR2* mutations have an autosomal dominant inheritance, whereas *CASQ2* mutations are usually autosomal recessive. Compared to RyR2, Casq2-linked CPVT is much less common, but is often more severe clinically [65]. On the other hand, contractile function of CPVT patients is surprisingly normal [61].

Experimental studies of Casq2-linked CPVT have been instrumental in defining the role of Casq2 in SR Ca^{2+} buffering and in regulating the RyR2 open probability [22, 41]. In particular, experiments in CPVT mouse models confirmed that the systolic function is preserved, and demonstrate that the higher arrhythmogenic risk in CPVT can be attributed to spontaneous Ca^{2+} releases from the SR during diastole [34]. However, the interactions between Casq2 and the other SR proteins regulating Ca^{2+} releases appear to be complex and many questions remain. Here, we review the physiology of intracellular Ca^{2+} handling and then focus on the pathophysiological mechanisms leading to CPVT caused by *CASQ2* mutations. Finally, we describe the clinical aspects of the disease and its contemporary therapeutic management.

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2 Physiology of cardiac Ca^{2+} handling

2.1 Excitation-Contraction coupling

Figure 1 illustrates the major protein complexes and cellular organelles involved in excitation-contraction coupling, which is the process of how an electrical signal, the cardiac action potential, generates a contractile response. In cardiac excitation-contraction (EC) coupling (Fig. 1), the cardiac cycle initiates by membrane depolarization due to ion entry through gap junction channels from a neighboring cardiomyocyte [36]. This initial depolarization opens voltage-gated Na^+ channels. The ensuing Na^+ current is responsible for the rapid upstroke (phase 0) of the action potential (AP). The membrane depolarization then opens voltage-gated L-type Ca^{2+} (Cav 1.2) channels located primarily in the transverse T-tubular membrane of cardiac myocyte (Fig. 1). Ca^{2+} entry through Cav 1.2 channels triggers Ca^{2+} induced Ca^{2+} release (CICR) [18], whereby a small influx of Ca^{2+} triggers a much bigger release of Ca^{2+} from the SR via activation of RyR2 Ca^{2+} release channels located in the terminal cisternae of the SR (Fig. 1). The released Ca^{2+} then binds to the troponin complex, activates contractile apparatus and the heart contracts. Contraction stops and the relaxation begins when SR Ca^{2+} release terminates and cytosolic Ca^{2+} concentration returns to its diastolic value. Ca^{2+} is removed from the cytosol by two main mechanisms: reuptake of Ca^{2+} into the SR via a Ca^{2+} -ATPase pump (SERCA2a) and extrusion from the cell via the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX). Also contributing albeit to a much smaller degree are the sarcolemmal Ca^{2+} -ATPase pump and mitochondrial Ca^{2+} uptake via the Ca^{2+} uniporter. Intracellular Na^+ homeostasis is reestablished by the Na^+/K^+ pump which extrudes Na^+ back into the extracellular space. Together, these mechanisms effectively reduce cytosolic Ca^{2+} ions, thereby promoting Ca^{2+} dissociation from the myofilaments and relaxation of the heart [7].

2.2 Casq2 and the cardiac Ca^{2+} release unit (CRU)

Casq2 is a glycoprotein that binds Ca^{2+} with high-capacity and low-affinity. Casq2 is found exclusively in the junctional sarcoplasmic reticulum (jSR) of cardiac myocyte [9, 10, 28]. Casq2 monomers can aggregate into dimers and then tetramers, which further increases Ca^{2+} binding capacity by producing negatively charged pockets that can bind up to 40 Ca^{2+} ions [57, 77]. This conformational change is dynamically regulated by the SR Ca^{2+} concentration, a process known as Ca^{2+} -induced polymerization [5, 59]. In this way Casq2 can effectively buffer the SR Ca^{2+} and usually maintains free Ca^{2+} concentrations below the inhibitory level of SERCA and, thereby promotes Ca^{2+} re-uptake into the SR [57].

Casq2 is anchored to the RyR2 via two proteins, junctin and triadin [29, 39], and together with the Ca^{2+} release channel RyR2, they form the part of the Ca^{2+} release unit located in the SR [84]. The CRU is responsible for Ca^{2+} release from the SR which is triggered by increase in cytosolic Ca^{2+} concentration due to activation of L-type Ca^{2+} channels (CICR) [16, 17]. In this way, the CRU provides precise control of Ca^{2+} release during the excitation-contraction coupling cycle in cardiac tissue [19]. In addition to serving as a Ca^{2+} buffer, Casq2 has been suggested to directly modulate activity of RyR2 [21, 27], presumably regulating its sensitivity to intra-SR luminal Ca^{2+} [40]. Triadin and junctin are considered as linking elements which mediate interactions between Casq2 and RyR2 [21, 71]. Studies suggest that triadin is also important for maintaining the structural and functional integrity of the cardiac CRU; and its ablation reduces SR Ca^{2+} release and impairs negative feedback of SR Ca^{2+} release on Cav1.2 Ca^{2+} currents [13]. More recently, junctin was reported to directly modulate RyR2 sensitivity to luminal Ca^{2+} [2]. Thus, in addition to Casq2, triadin and junctin should be considered as candidate genes in CPVT, although no CPVT mutations have been identified to date.

3 Calsequestrin 2 mutations

3.1 Human genetics

CPVT is a severe inherited arrhythmia disorder characterized by adrenergically mediated ventricular tachycardia caused by altered function of proteins composing the CRU [43, 63]. The estimated prevalence of CPVT in the population is around 1:10000 [58]. So far two forms of the disease have been described: one form is associated with dominant mutations in the *RYR2* gene [63, 64] and is responsible of 50-55% of all CPVT cases [54]. The other form is due to recessive mutations in the *CASQ2* gene, which resides on the short arm of chromosome 1 [43, 61] and accounts for only 3-5% of the CPVT patients [1]. Presently, 15 *CASQ2* mutations have been identified in the short arm of chromosome 1: 3 common polymorphisms (not associated with disease) and 12 missense, deletion and nonsense mutations (Table 1). Interestingly, all mutations studied to date lead to either a severe reduction or complete loss of Casq2 protein [66, 69]. This observation may explain why the Casq2-linked CPVT phenotype is rather homogeneous among patients with different mutations. Heterozygous *CASQ2* mutations cause approximately 25% reduction of functional protein and do not lead to clinical CPVT per se [12]. However, based on the modest but measureable increased arrhythmia risk in heterozygous *Casq2* null mice [12], heterozygous carriers of *CASQ2* mutations may be more susceptible to ventricular arrhythmia in the presence of comorbidities affecting intracellular Ca^{2+} handling.

Important new information on the pathophysiology of human *CASQ2* mutations has been obtained from gene-targeted mouse models (Table 1) [12, 34, 66, 69]. The mice largely reproduce the clinical characteristics of Casq2-linked CPVT patients such as resting bradycardia and stress-induced polymorphic and bi-directional ventricular tachycardia (Fig. 2), in the presence of a preserved contractile function [34]. The study of these mouse models has provided new insight into the alterations of the SR ultrastructure and SR protein levels caused by *Casq2* mutations, clarified the physiological role of Casq2 in the CRU, and how the reduction or absence of this protein alters cardiac Ca^{2+} handling and causes CPVT [34]. Moreover, the *Casq2* mutant mouse models have been used as tools for in vitro and in vivo drug testing [26].

4 CPVT pathophysiology

4.1 Cellular mechanisms

At rest, cardiac contractility and systolic SR Ca^{2+} release is unchanged in *Casq2* mutant myocytes [34]. Premature SR Ca^{2+} release occurs in conditions of SR Ca^{2+} overload, such as exposure to catecholamines that stimulate β -adrenergic receptors. Mechanistically, RyR2 channels that lack Casq2 open spontaneously without being triggered by voltage-gated Ca^{2+} influx. One of the factors that potentially increases the pro-arrhythmic phenotype is accelerated recovery of SR Ca^{2+} release refractoriness. Functionally, Ca^{2+} entering through Cav 1.2 during an action potential triggers Ca^{2+} release from the SR. The amplitude of the resulting Ca^{2+} transient depends on the diastolic interval that preceded the second stimulus (the amplitude is lower when the diastolic interval is short) [35], a fundamental mechanism known as Ca^{2+} transient restitution. The underlying mechanism(s) are not fully understood, but several processes are likely involved in the SR Ca^{2+} release refractoriness including restitution of the triggering L-type Ca^{2+} current (Cav 1.2 channel), RyR2 channel refractoriness and SR Ca^{2+} uptake kinetics. A recent report suggests that Casq2, acting both as intra-SR Ca^{2+} buffer and as a regulator of sensitivity of RyR2 to luminal Ca^{2+} , importantly regulates Ca^{2+} transient restitution [40]. On one hand, loss of Casq2 results in a faster rise in intra-luminal free Ca^{2+} after each heart beat in the *Casq2* null mouse model, making more Ca^{2+} available for release in case of premature (spontaneous) activation of RyR2. On the other hand, larger SR Ca^{2+} release can be triggered at the same free intra-SR

Ca^{2+} concentration, which confirms the direct inhibitory role of Casq2 on SR Ca^{2+} release independently of Casq2's role as a Ca^{2+} buffer. These data are also supported by studies in cardiomyocytes overexpressing Casq2 that show a significant reduction of both Ca^{2+} -induced and spontaneous Ca^{2+} release despite the much higher total SR Ca^{2+} load [30]. Taken together, these results demonstrate that Casq2 plays crucial role in Ca^{2+} transient restitution via complex regulation of SR Ca^{2+} release refractoriness in cardiac muscle.

At the same time, it is unclear if this impairment in RyR2 regulation is attributable to reduction of Casq2 itself or to the proteins that mediate binding of Casq2 to RyR2 channel (namely, triadin and junctin) [21]. Considering that almost all *Casq2* mutant mouse models (including those with Casq2 overexpression) reported to date exhibit significant reduction of these Casq2-binding proteins and that mouse models with gene-targeted deletion of triadin or junctin also generate an arrhythmogenic phenotype in the absence of *Casq2* mutations [13, 83], it is possible that reduction of triadin and junctin may independently contribute to the arrhythmia risk associated with *Casq2* mutations [37]. Other factors such as changes in expression levels of RyR2, calreticulin and histidine-rich Ca^{2+} binding protein (HRC) [23, 56, 69] and SR volume remodelling [34] observed in some but not all *Casq2* mutant mouse models may also be responsible for CPVT. The relevance of these findings to the pathogenesis of CPVT remains to be determined.

4.2 Generation of ectopic triggered activity

A consistent finding in experimental models of *RyR2* or *Casq2* mutations is an increased diastolic Ca^{2+} leak from the SR that results in a high frequency premature spontaneous SR release [34, 63]. The β -adrenergic stimulation present during physical or emotional stress further increases the rate and magnitude of these spontaneous Ca^{2+} releases by loading the SR with Ca^{2+} [32]. Raising the cytosolic Ca^{2+} concentration activates the electrogenic NCX exchanger on the cell surface, generating an inward Na^{+} current that depolarizes the cell membrane during diastole [7]. Such an event is termed delayed after depolarization (DAD), which has been proposed to induce triggered activity and life threatening ventricular arrhythmias [79]. However, to overcome the electrical sink provided by electrically-coupled neighbouring cells, many synchronized DADs in contiguous excitable cells are necessary to generate an impulse that propagates to the entire heart [82]. In addition, a number of studies have investigated the cell type responsible for the generation of the arrhythmic events. Most of the results so far obtained on CPVT mouse models point towards the Purkinje fibers of the specialized conduction system as the cell type responsible for arrhythmia initiation in CPVT [11, 31]. The bi-directional ventricular tachycardia (Fig. 2) characterized by alternating QRS axis shifts and typical of CPVT syndromes has been explained as arising alternatively from the right and left bundle branch, or from hemibranches of the same side [4, 55]. Most recently, computer-based CPVT models have supported this “ping-pong” mechanism and the likely role of Purkinje fibers in CPVT, due to their Ca^{2+} handling characteristics that promote spontaneous Ca^{2+} release and triggered activity [4, 47]. Although the definite proof is still elusive, the collective data suggest that Purkinje cells could be targeted to treat CPVT [31].

5 Clinical aspects

5.1 Clinical phenotype and diagnosis

Despite the range of *CASQ2* mutations described in the literature, the clinical presentation of the Casq2-linked CPVT is rather homogeneous. While the age of onset of the first symptoms may vary somewhat, patients are usually diagnosed in their first or second decade of life. The subjects usually manifest recurrent stress-induced palpitations, syncopal episodes or seizures, for which they seek medical assistance [44, 61]. However, establishing

the diagnosis can be challenging. During the diagnostic work-up cardiac imaging does not reveal any macroscopic structural abnormality. The resting ECG is usually non-diagnostic, with PR and QT intervals within normal limits (Fig. 2a). A mild sinus bradycardia, often observed in CPVT patients, may be the only hint towards the disease [62]. When present, a family history of syncope, cardiac arrhythmias or sudden death suggests a genetic disorder and should prompt further investigation. The gold standard for CPVT diagnosis is the exercise stress-test (Fig. 2). Alternatively, a pharmacological stress-test can be done [58]. Patients usually display sporadic premature beats at the beginning of the stress (Fig. 2b) that progress into more complex arrhythmias such as bigeminy, couplets and non-sustained ventricular tachycardia (Fig. 2c) as the exercise continues and more catecholamines are released [44, 64]. If the exercise is not stopped, ventricular tachycardia can become sustained and eventually lead to ventricular fibrillation and cardiac arrest.

Characteristic of CPVT is a bi-directional VT (Fig. 2c), so called because of the alternating QRS axis of the ectopic beats. However, bi-directional VT is not a pathognomonic feature as it can be observed also in other conditions such as myocarditis, digitalis toxicity, and myocardial ischemia as well as a phenotypically similar but genetically-distinct condition, Andersen-Tawil Syndrome [8, 20, 52, 70]. Perhaps less appreciated is the potential for coexisting atrial arrhythmias in CPVT patients. Interestingly, most patients are asymptomatic during bidirectional VT and symptoms are usually attributable to episodes of rapid, polymorphic VT or ventricular fibrillation. Once a CPVT patient has been identified, first degree relatives of the proband should be screened with 24h Holter ECG recording and/or exercise-stress test and referred for genetic evaluation to help identify mutations carriers and early diagnose potential patients with milder or atypical phenotype.

5.2 Treatment

5.2.1 Conventional therapies—Since CPVT arrhythmias are provoked by catecholamine discharge in response to exertion or emotional stresses, β -blockers have long been considered as the most appropriate treatment [48, 62]. When the use of β -blockers is contraindicated or in patients symptomatic at maximum tolerated dose of β -blockers, Ca^{2+} channel blockers such as verapamil have been used [67, 74]. However, the efficacy of Ca^{2+} channel blockers is not clear. Furthermore, despite reducing the frequency of arrhythmic events in CPVT patients, β -blockers fail to prevent sudden cardiac death in all cases [6, 64, 72]. Up to 30% of patients require interventional approaches such as implantable cardioverter defibrillators (ICD) [24] and left sympathetic denervation [3, 80]. However, these therapies are invasive and costly, and are not without risk [53, 60, 62]: For example, appropriate ICD discharge in CPVT patients produces a catecholamine surge and recurrent tachycardia prompting repeated shocks; the ensuing electrical storm may prove lethal [53, 60, 62]. When performed properly, left sympathetic surgical ablation can reduce, but may not eliminate events. In addition, such invasive approaches can have a major negative impact on the quality of life in young CPVT patients.

5.2.2 New pharmacological approaches—During the last few years, basic and translational research studies have provided new knowledge about the pivotal role of Ca^{2+} -handling abnormalities and RyR2/Casq2 dysfunction in CPVT pathophysiology. This led to the identification of the RyR2 as an important drug target for treating CPVT. For example, Watanabe et al. found that flecainide, a class I anti-arrhythmic drug, blocks RyR2 channels and holds promise as a new treatment for CPVT [78]. Flecainide effectively reduced exercise-induced VT in CPVT mouse models, most likely due to a dual effect on the Na^{+} channel and the RyR2 channel: In isolated myocytes, flecainide reduced both the rate of spontaneous Ca^{2+} releases and triggered action potentials [25, 50, 78]. Initial clinical data in 33 CPVT patients refractory to standard therapy with β -blockers and Ca^{2+} -blockers were

promising, with a >70% response rate after flecainide was started [76]. A randomized prospective clinical trial of flecainide versus placebo is currently being conducted to further test flecainide efficacy and safety in CPVT on a larger scale. Similarly, propafenone, another class I anti-arrhythmic drug, also blocks RyR2 channels and reduces CPVT incidence both in a mouse model and CPVT patients. It may represent an alternative to flecainide in countries where flecainide is not available [26]. In 2011, Zhou et al. discovered that the β/α 1-blocker carvedilol inhibits RyR2 channels by open state block analogous to flecainide and suppressed spontaneous Ca^{2+} release in a CPVT mouse model. Not only was carvedilol more effective than other β -blockers, but analogs of carvedilol without any β -blocking properties still suppressed CPVT mouse models, emphasizing the emerging concept that RyR2 inhibition is important for treating CPVT [38, 85]. Experimental inhibitors of Ca^{2+} /calmodulin-dependent protein kinase II have been shown to suppress spontaneous Ca^{2+} release events and prevent arrhythmias in a RyR2-mutant mouse model of CPVT, likely via modulating several aspects of Ca^{2+} handling [49]. Other experimental agents that reduce RyR2 open probability, and inhibit SR Ca^{2+} leak such as S107 and JTV519 have also been effective against Ca^{2+} triggered arrhythmias [45, 46, 68]. An S107-derivative is currently in early phase clinical trials for CPVT (Table 2).

6 Summary and conclusions

Although CPVT caused by *CASQ2* mutations is a very rare clinical syndrome, the study of experimental models of *Casq2* mutations has generated a wealth of important new mechanistic insight on the role of abnormal cardiac Ca^{2+} handling in ventricular arrhythmias and sudden death. Moreover, the experimental work in *Casq2* mutant mouse models has led to new drug therapies that have already been translated into clinical care and are likely to benefit CPVT patients for years to come.

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Fig. 1. Cardiac myocyte. Illustrated are ventricular myocyte architecture, intracellular organelles and protein complexes involved in excitation-contraction coupling and the pathogenesis of Ca^{2+} triggered ventricular arrhythmias caused by *Casq2* mutations

Fig. 2. ECG recordings during an exercise stress-test in a CPVT patient and a pharmacological stress test in a *Casq2*^{-/-} CPVT mouse model. a) Sinus rhythm at the beginning of the test, b) occurrence of sporadic premature beats and couplets (red arrows), c) bi-directional VT

Table 1*CASQ2* mutations in humans and available mouse models

Mutation type	Human mutation	Human phenotype	Mouse model of human mutation	Characteristics of mouse model
missense	D307H[42]	CPVT	Homozygous knock-in (<i>Casq2</i> D307H)[69]	VE at rest, severe CPVT, LVH in aged mice, Young mutant mice had structurally normal hearts but stress-induced ventricular arrhythmias; aging produced cardiac hypertrophy and reduced contractile function
missense	R33Q[75]	CPVT	Homozygous knock-in (<i>Casq2</i> R33Q)[66]	Ventricular arrhythmias at rest, develop bidirectional VT on exposure to environmental stress
non sense	R33X[61]	CPVT	Homozygous knock out[34, 69]	Severe CPVT, very mild LVH, normal contractile function, slight increase in heart weight and left-ventricular wall thickness
splicing	532+1 G>A[61]	CPVT		
deletion	L23fs+14X[61]	CPVT		
deletion	G112+5X[15]	CPVT		
	Any heterozygous <i>CASQ2</i> mutation	Unclear, possibly not clinically relevant	Heterozygous knock-out[12]	Very mild CPVT: catecholamergic challenge and programmed stimulation induced significantly more ventricular ectopy in <i>Casq2</i> ^{+/-} mice than in <i>Casq2</i> ^{+/+} mice
missense	L167H[15]	CPVT as compound heterozygous with G112+5X		
missense	Y55C[14]	CPVT as compound heterozygous	-	-
missense	P308L[14]			
missense	F189L[51]	CPVT	-	-
missense	E177Q[81]	Not characterized	-	-
missense	K206N[33]	Unclear	-	-
SNP	T66A[81]	(NCR)		
SNP	V76M[81]	(NCR)	-	-
SNP	D395D[81]	(NCR)	-	-

Table 2

Contemporary and investigational CPVT therapy

	Target	Mechanism of action	status
CONVENTIONAL PHARMACOLOGICAL THERAPY			
β -adrenergic antagonists	β 1-adrenergic receptor	Reduce pro-adrenergic effects of circulation catecholamines and synaptic neurotransmitters	Routine clinical use
Ca^{2+} -channel blockers	L-type Ca^{2+} channel	Decreases heart rate, reduces the flux of Ca^{2+} into the cell	Routine clinical use
INTERVENTIONAL THERAPY			
Implantable cardioverter defibrillator (ICD)		Delivers an electrical shock to resets the intrinsic sinus rhythm when an arrhythmia is detected	Case studies
Sympathetic denervation		Reduces nerve-mediated adrenergic stimulation on the heart	Case studies
NEW PHARMACOLOGICAL APPROCHES			
Flecainide	Na^+ channel and RyR2	Blocks RyR2 channel in open state configuration, reduces the rate of spontaneous Ca^{2+} releases	Ongoing clinical trial
Propafenone	Na^+ channel and RyR2	R-enantiomer blocks RyR2 channel in open state configuration, reduces the rate of spontaneous Ca^{2+} releases	Case studies
Carvedilol and analogs	RyR2	Reduces the open-state duration of RyR2 Ca^{2+} release channels	Preclinical
S107 and analogs	RyR2	Reduces RyR2 channel openings possibly via the RyR2 modulating protein calstabin2	Ongoing clinical trial
JTV519 (K201)	RyR2	Reduces RyR2 channel openings possibly via the RyR2 modulating protein calstabin2	Preclinical
Calmodulin kinase II inhibitors	Multiple ion channels and transporters	Reduces cellular Ca^{2+} loading and RyR2 channel activity	Preclinical