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Ryanodine Receptor Channelopathies: The New Kid in the Arrhythmia Neighborhood

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1. Introduction

Cardiac arrhythmia is a major mortality cause in both acquired and inherited cardiomyopathy, accounting for more than 750.000 deaths per year (~ 0.1% of total recorded deaths) in Europe and the USA (Priori SG, 2002). The 2008 WHO rapport has foreseen that cardiovascular disease will be the world leading death cause in the near future, surpassing infectious diseases.

Some of these cardiac diseases are acquired as cardiac hypertrophy, which develops as an adaptation of the heart to diseases that challenge the heart work chronically. Cardiac hypertrophy often degenerates in heart failure (HF), the final outcome of most cardiovascular diseases. Chronic HF prevalence is increasing in western countries, with only 25% of men and 38% of women surviving 5 years after the onset of clinical signs. Quality of life is hampered by the reduced pump function, which can also lead to death. However, half of deceases in HF patients are sudden due to cardiac arrhythmia. During cardiac pathology, altered activity of the cardiac, type 2, ryanodine receptor (RyR2) may generate arrhythmia and sudden death. This risk is high in HF where there is a profound remodeling of Ca^{2+} cycling, and alterations in transmembrane Ca^{2+} influx, Ca^{2+} release or/and sarcoplasmic reticulum (SR) Ca^{2+}-load underlie systolic dysfunction (Gómez *et al.*, 1997; Bénitah JP, 2002). Thus, when dealing with HF and poor cardiac outcomes, it is a need to better understand the mechanisms of cardiac arrhythmia in order to efficiently treat these patients. However, a large number of inherited arrhythmogenic syndromes that cause sudden death have been characterised. Some are associated with structural heart disease, such as familial hypertrophic cardiomyopathy and arrythmogenic right ventricular cardiomyopathy type 2 (ARVD2). Others do not produce structural heart disease and so are difficult to detect. Most of these cardiomyopathies are due to mutations in plasmalemmal cardiac ion channels, mainly the Na^+ channel and several K^+ channels (Lehnart *et al.*, 2007). These mutations promote arrhythmogenesis by altering the action potential (AP) duration, which therefore may enhance the propensity of arrhythmic activity via the development of early after depolarizations (EADs). However, the recent finding of mutations in the Ca^{2+} release channel (RyR2) associated with catecholaminergic polymorphic ventricular tachycardia

(CPVT) and ARVD2 has opened a new view of arrhythmogenesis, evidencing that alterations in intracellular Ca^{2+} cycling can generate arrhythmia (Priori SG, 2001). CPVT is a familial arrhythmogenic disorder characterised by syncopal events and Sudden Cardiac Death occurring in children and young adults during physical stress or emotion in the absence of structural heart disease. In addition to the severe phenotype, CPVT exhibits a cumulative mortality of 30 – 50% by 35 years. To date, more than 145 RyR2 mutations have been identified as causative of CPVT in affected individuals, which appear clustered in 3 "hot spots". Some of these mutations have been investigated in several *in vitro* systems (lipid bilayers, HEK293 cells, HL1-cardiomyocytes), suggesting that CPVT-linked RyR2 mutations produced an increase of the RyR2 activity, termed as RyR2 Ca^{2+} leakage, under beta-adrenergic stimulation (Lehnart *et al.*, 2004). This abnormal SR Ca^{2+} release during diastole would activate the Na^{+}-Ca^{2+} exchanger (NCX) to extrude Ca^{2+} out of the cell. Since NCX is electrogenic, a net inward current is generated for each Ca^{2+} ion extruded, which could develop delayed after depolarizations (DADs) and evoke triggered activity if they reach threshold. This abnormality may promote arrhythmogenesis in CPVT patients, where the increased RyR2 activity may generate DADs through the activation of NCX (Nakajima *et al.*, 1997). This mechanism is interestingly very similar to the one that has been suggested in HF, where chronic hyperadrenergic state generates an inadequate diastolic Ca^{2+} release (Ca^{2+} leak) and SR Ca^{2+} depletion, leading to a decreased myocardial contractility. Recently, Priori's laboratory has developed a knock-in mouse model carrying a highly penetrant R4496C mutation in the RyR2 (equivalent to the human R4497C mutation), identified in an Italian family with CPVT. Previous reports have shown that these mice developed bidirectional and polymorphic ventricular tachycardia under the injection of isoproterenol (β-adrenergic agonist) and caffeine (Cerrone *et al.*, 2005). Interestingly, the presence of DADs was detected after high pacing rates and under the application of isoproterenol, in isolated ventricular myocytes (Liu *et al.*, 2006). Our laboratory has also performed experiments using this mouse model and, in addition to other findings, we observed abnormal cytosolic Ca^{2+} release and spontaneous triggering activity, in ventricular myocytes paced at high rates or treated with isoproterenol (Fernandez-Velasco M, et al 2009). In this chapter, we will review the latest knowledge on the role of intracellular Ca^{2+} on cardiac arrhythmia in acquired and inherited diseases, paying special attention to the molecular and cellular mechanisms of the disease.

2. Involvement of RyR in cardiac arrhythmias

The cardiac RyR is the major Ca^{2+} release channel in the ventricle and it is central in activating contraction by the mechanism of Ca^{2+} -induced Ca^{2+} release during the excitation-contraction process. It is located in the membrane of the SR, mainly in the junctional SR, facing the L-type Ca^{2+} channels located in the membrane invaginations termed trasverse tubules. During cardiac excitation-contraction coupling (ECC), the membrane depolarization during the AP activates Ca^{2+} influx via sarcolemmal L-type Ca^{2+} channels, providing enough Ca^{2+} to activate the RyR (Fabiato, 1983; Bers, 2002). By this mechanism, the initial Ca^{2+} signal is greatly amplified, then providing enough Ca^{2+} for contraction. Relaxation occurs when calcium is removed from cytosol, mainly by NCX and SR Ca^{2+}-ATPase (SERCA). The sarcolemmal Ca^{2+} ATPase, different from SERCA, can also extrude some Ca^{2+}. However, its contribution appears to be minor (about 3% of total Ca^{2+} removal) and its physiological significance has yet to be determined. For equilibrium to occur, the amount of Ca^{2+} extruded through the NCX should be equivalent to the amount of Ca^{2+} entering the cell through DHPRs, and the amount of Ca^{2+}

transported by SERCA should be equivalent to Ca^{2+} released by the SR. For each Ca^{2+} extruded, the NCX enters 3 Na^{+}, generating thus an inward current. In cases of Ca^{2+} overload, spontaneous SR Ca^{2+} release through RyRs produces Ca^{2+} waves, activating transient inward currents (Iti), (Berlin *et al.*, 1989) which if they reach threshold may trigger an action potential (triggered activity). The NCX is centrally involved in this current (Venetucci *et al.*, 2007). Triggered activity-derived arrhythmias are produced by after depolarizations that can occur early during the repolarization phase of the action potential (early after depolarization, EAD) or late, after completion of the repolarization phase (delayed after depolarization, DAD) (Figure 1). When either type of after depolarization is large enough to reach the threshold potential for activation of a regenerative inward current, a new AP is generated, which is named as triggered activity.

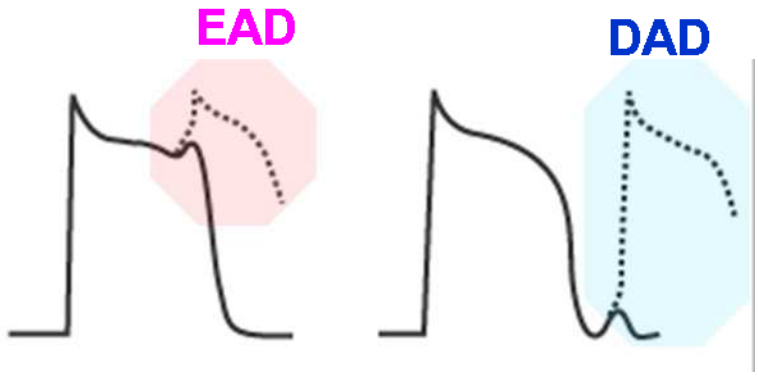

Fig. 1. Example of an early after depolarization (EAD) and delayed after depolarization (DAD) leading to triggered activity.

3. Involvement of RyR in cardiac arrhythmias in the hypertrophied and failing heart

Half of the deaths in heart failure patients are due to sudden death and cardiac hypertrophy has also an elevated risk of sudden death, which may arise as a consequence of ventricular arrhythmia. The ventricular cardiomyocytes of these hearts are prolonged, which favors the occurrence of early EADs. EADs are believed to be dependent on the L-type Ca^{2+} channel. DADs are common of Ca^{2+} overloaded cells, which is uncommon in heart failure. However, the RyR activity is altered, which may favor DADs. Several modifications that happen in the RyR of the failing hearts have been shown to promote diastolic Ca^{2+} leak and be arrhythmogenic. These modulations include phosphorylation (PKA and/or CaMKII), oxidation, decreased nitrosylation, lost of its accessory protein FKBP12.6 (calstabin 2) and unzipping. However, the RyR leakiness would not be enough to provoke arrhythmia, because it should be compensated by the reduction in SR Ca^{2+} load (Venetucci *et al.*, 2008). Hyperphosphorylation of RyR by PKA was found in humans at end stage heart failure and different models of heart failure (Marx *et al.*, 2000; Reiken *et al.*, 2003) and related to sudden death (Marks, 2001), although controversed (Jiang *et al.*, 2002). RyR phosphorylation increases activity of RyR, making RyR leaky and thus favoring arrhythmia. The RyR is also hyperphosphorylated at the CaMKII site (Ai *et al.*, 2005) during heart failure, which may be

involved in the propensity to arrhythmias. In this sense, CaMKII blockade repressed the spontaneous Ca^{2+} waves in heart failure cardiomyocytes (Curran *et al.*).

RyR phosphorylation in heart failure has been suggested to unbind the RyR from its regulatory protein, the FKBP12.6 (Marx *et al.*, 2000). While the direct correlation with phosphorylation is a matter of debate (Maier *et al.*, 2003; Blayney *et al.*, 2010), the cardiac expression of FKBP12.6 is reduced in heart failure, causing diastolic Ca^{2+} leak that may result in higher propensity of DADs and consequent triggered arrhythmias (Shou *et al.*, 1998; Yano *et al.*, 2000; Reiken *et al.*, 2001; Xin *et al.*, 2002; Wehrens *et al.*, 2004; Ai *et al.*, 2005; Wehrens *et al.*, 2005; Yano *et al.*, 2005; Huang *et al.*, 2006; Yano *et al.*, 2006; Gomez *et al.*, 2009). Supporting the role of FKBP12.6 in arrhythmia, stabilizing FKBP12.6 binding to RyR by FKBP12.6 overexpression prevents triggered arrhythmias in normal hearts, probably by reducing diastolic SR Ca^{2+} leakage (Gellen *et al.*, 2008). Conversely, FKBP12.6 knockouts exhibited exercise induced ventricular arrhythmia (Wehrens *et al.*, 2003).

Other alterations during heart failure may affect RyR function. In this sense, RyR oxidation in a canine model of sudden death are involved in arrythmogenic $[Ca^{2+}]_i$ transients alternans (Belevych *et al.*, 2009). Moreover, the increase in xantine oxidase activity also reduces the level of S-nytrosilation. The RyR hyponitrosylation has also been involved in the Ca^{2+} leak from SR in experimental heart failure (Gonzalez *et al.*, 2010). This alteration might contribute to the arrhythmogenesis in heart failure. In this sense, it has been shown that NOS1-/- mice show RyR hyponitrosylation with consequent SR Ca^{2+} leak and an arrhythmic phenotype, without altering the FKBP12.6 stoichiometry (Gonzalez *et al.*, 2007). Consistent with these findings, NOS1 overexpression protected the mice in a model of heart failure by preserving Ca^{2+} cycling (Loyer *et al.*, 2008). However, others have found that RyR is hypernitrosilated in a model of muscular dystrophy, where arrhythmias are frequent, suggesting that hypernytrosilated RyR is leaky. It should be noted that in this model the binding to FKBP12.6 was also decreased (Fauconnier *et al.*, 2010), which may account for the RyR leakiness.

The N and central domains of the RyR interact with each other in a process called "zipping", which stabilizes the channel in its closed state (Ikemoto & Yamamoto, 2002). In heart failure, the RyR is unzipped favoring its phosphorylation and unbinding to FKBP12.6 (Oda *et al.*, 2005).

Besides these direct alterations of the RyR, this channel may be "sensitized" in some conditions by an increase in the local $[Ca^{2+}]$ around it, from either side of the SR membrane. In this sense, the increase in the IP3R expression in the junctional SR during heart failure might, under certain circumstances, locally increase the $[Ca^{2+}]_i$ in the neighboring RyRs and facilitate Ca^{2+} waves propagation (Harzheim *et al.*, 2009). Increasing the $[Ca^{2+}]_i$ in the luminal side, as by an increase in SERCA activity, might also sensitize the RyR and participate in Ca^{2+} waves formation (Keller *et al.*, 2007) although SERCA activity is thought to be depressed in heart failure.

4. Involvement of RyR in inherited cardiac arrhythmias

4.1 Catecholaminergic polymorphic ventricular tachycardia

CPVT is a rare arrhythmogenic disease characterized by exercise or stress induced ventricular tachyarrhythmia, syncope, or sudden death that appear in individuals with

structural normal hearts (Leenhardt *et al.*, 1995; Coumel, 1997; Priori *et al.*, 2002). Because the electrocardiogram (ECG) of CPVT patients is unremarkable under basal conditions, the diagnosis is established in symptoms and the detection of stress-induced arrhythmias during exercise test or Holter recording. Although some CPVT patients develop polymorphic ventricular tachycardia (VT), the bidirectional VT is considered the diagnostic marker of CPVT (Priori *et al.*, 2002). Interestingly, bidirectional VT occurs during digitalis intoxication, where the Na^+/K^+ ATPase pump is inhibited, increasing the intracellular Na^+ concentration that in turn, by NCX induces an intracellular Ca^{2+} overload, triggering arrhythmogenic DADs (Rosen & Danilo, 1980). Thus, it was reasonable to postulate that bidirectional VT in CPVT patients can be due to changes in the intracellular calcium handling. Indeed, several reports have associated CPVT with mutations in genes encoding key-proteins involved in the control of intracellular calcium handling, such as RyR2 and calsequestrin (CASQ2), causative of CPVT1 and CPVT2, respectively (Lahat *et al.*, 2001a; Priori *et al.*, 2002).

4.2 RyR2 mutations in CPVT

The gene encoding RyR2 (chromosome 1q42.1–43) consists of 105 exons, which encodes 4967 amino acids (~560 kDa) and it is one of the largest and most intricate in the human genome. The RyR2 is a homotetramer with hydrophobic segments of the four identical subunits forming a central Ca^{2+} pore (Wagenknecht, 1989). Currently more than 145 RyR2 mutations have been reported as causative of CPVT, and they continue growing since the first mutations was reported a decade ago (Priori SG, 2001) – an updated database is shown in the 'Gene connection for the heart' website (http://www.fsm.it/cardmoc/). Some of these RyR2 mutations have been identified in patient groups screened for Long QT syndrome (Tester DJ, 2005), and ARVD2 (Tiso *et al.*, 2001). CPVT-related arrhythmias are by far reproduced during an exercise stress test, by isoproterenol infusion, or by other forms of adrenergic stimulation (Sumitomo *et al.*, 2003; Vyas *et al.*, 2006). A genetic screening of RyR2 is necessary to verify the disease in patients suspicious of CPVT1, although this strategy is time consuming and expensive. However, screening for RyR2 mutations could be simplified due to the circumstance that CPVT mutations used to cluster in certain exons, and a tiered scan of these exons can be used to lower the cost (Medeiros-Domingo A, 2009). RyR2 mutations linked to CPVT are clustered into 3 discrete protein regions or "hot spots": N-terminus (32%), central domain (30%) and C-terminus (38%) (Yano *et al.*, 2006; George CH, 2007). Similar mutation clustering is observed in the *RYR1* gene, which encodes the skeletal muscle RyR1 and is linked to malignant hyperthermia and central core disease (Dirksen, 2002). The N-terminus (also called domain I: amino acids 77-466) is a domain particularly susceptible to conformational change. It contains the cytoplasmic loop, which it is postulated that interacts with the central domain (zipping) stabilizing RyR2 activity during diastole (Yamamoto T, 2000). The central domain (domain II: amino acids 2246-2534) contains an FKBP12.6 binding domain (1636–1937) and it is supposed to interact with the N-terminus domain (zipping-unzipping). The C-terminus domain (domain III: amino acids 3778-4201 and domain IV: amino acids 4497 to 4959) contains the transmembrane regions of the Ca^{2+} channel and an hydrophobic region which it is postulated to transduce cytoplasmic events to regulate the Ca^{2+} pore forming domain (George CH., 2006). Only a small number of mutations are located in regions of RyR2 outside these portions. By contrast to other

channelopathies, most of the RyR2 mutations described in CPVT are single nucleotide replacements ("point mutations") leading to an amino acid substitution.

4.3 Functional alterations of CPVT related mutations in the RyR

Although the phenotypic manifestation of CPVT is usually the stress-induced development of bidirectional or polymorphic ventricular tachycardia, patient symptoms are heterogeneous, presenting in some cases high variability among affected subjects within the same family (d'Amati & King, 2005). However in other cases, patients with point mutations located in the same RyR2 cluster present similar CPVT symptoms, probably because they affect RyR2 function in a common way. To improve the current knowledge of RyR2 complexity and to provide an adequate treatment to CPVT patients, it is necessary to study the molecular mechanisms of all sudden cardiac death (SCD)-linked mutations screened. A number of studies on them have been undertaken. Most of them have been analyzed in heterologous systems, but some transgenic mice have been constructed, allowing the exploration of the cardiac function.

CPVT-linked mutations have been expressed in various heterologous systems (lipid bilayer, HEK293 cells, HL1-cardiomyocytes). Specifically, HEK293 cells (human embryonic kidney cell line) have been widely used as an expression system. This cell line presents some weak points such as it lacks ECC proteins and the contractile machinery that characterizes heart cells. However, it presents several advantages: 1) it is easy to transfect using conventional methods and, 2) as it does not express native RyR, they cannot interfere with expressed constructs.

Some human N-terminus mutations (R176Q/T2504M and L433P), central domain mutations (S2246L and R2474S), and C-terminus mutations (N4104K, Q4201R, R4496C, I4867M and N4895D) have been explored using HEK293 cells. These RyR2 mutations showed an increased frequency of spontaneous Ca^{2+} oscillations and a reduced Ca^{2+} store content, thus displaying gain-of-function (Jiang *et al.*, 2004; Jiang D, 2005). In addition, most mutated RyR2 incorporated into lipid bilayers displayed an increased sensitivity to luminal Ca^{2+}, although the two N-terminus mutants were 10-fold less sensitive than the others (Jiang D, 2005). However, some RyR2 mutants displayed an increased sensitivity to cytosolic Ca^{2+} and caffeine. This is the case for S2246L, N4104K and R4497C mutations expressed in HEK293 cells or HL-1 cells, where it was also shown a gain-of-function RyR2 activity, while there was no change in SR Ca^{2+} load (George CH., 2006). In some cases, differences in RyR2 mutants' response to agonists are closely dependent on the mutational locus. This may be the case of a report from Thomas and coworkers, who have observed marked differences in caffeine-dependent Ca^{2+} release in N-terminal and central domain ARVD2-linked RyR2 mutations (L433P, N2386I and R176Q/T2504M) (Thomas *et al.*, 2004; George CH & .2005). Interestingly, one of these 3 mutations (L433P) was not associated with gain-of-function, but rather with loss-of-function (George CH & . 2005).

It is of note that the characterization of RyR2 mutations according to the mutational locus may be of large interest because this permits to design a model which integrates domain-specific arrhythmogenic mechanisms. The result model could extrapolate how new mutations may affect RyR2 function, allowing for a common therapy that restores channel activity. Table 1 shows a classification of SCD-linked mutations characterized so far.

Location in RyR2	Mutation	Disease	Characterization	System	RyR2 defect
N-Terminal	R176Q	ARVD / SUO	Enhanced SOICR increased sensitivity to luminal Ca^{2+} (Jiang D, 2005); R176Q/T2504M increase caffeine-dependent sensibility to cytosolic Ca2+ (Thomas, 2005); myocytes elicited oscillatory Ca^{2+} signals under β-adrenergic stimulation. (Kannankeril *et al.*, 2006)	HEK293 Lipid bilayers Knock-in mice	Gain-of-function
N-Terminal	E189D	CPVT	Increases the propensity of SOICR and enhance caffeine sensitivity. (Jiang D, 2010)	HEK293	Gain-of-function
N-Terminal	G230C	CPVT	Increased sensibility to cytosolic Ca^{2+}, decreased FKBP-12.6 binding (Meli, 2011)	HEK293 Lipid bilayers	Gain-of-function
N-Terminal	L433P	ARVD	Enhanced SOICR, increased sensitivity to luminal Ca^{2+} (Jiang D, 2005) Decrease caffeine-dependent, sensibility to cytosolic Ca^{2+} (Thomas, 2005), desensitized response to caffeine (Thomas *et al.*, 2004)	HEK293 Lipid bilayers HEK293	Gain-of-function Loss-of-function
Cytoplasmic loop	G1885E	ARVC	Enhanced SOICR, reduced RyR2 activity in G1885E/G1886S double mutant. (Koop, 2008)	HEK293	Gain-of-function
Cytoplasmic loop	G1886S	ARVC	Enhanced SOICR, reduced RyR2 activity in G1885E/G1886S double mutant. (Koop, 2008)	HEK293	Gain-of-function
Cytoplasmic loop	S2246L	CPVT/IVF	PKA-dependent increased RyR2 activity (Wehrens *et al.*, 2003); PKA and caffeine-dependent increased RyR2 activity (George *et al.*, 2003);abnormal domain interaction (George CH., 2006);enhanced SOICR, increased sensitivity to luminal Ca2+ (Jiang *et al.*, 2004)	HEK293 Lipid bilayers CHO HL-1	Gain-of-function
Cytoplasmic loop	R2267H	CPVT	PKA-dependent increased sensitivity to cytosolic Ca^{2+} (Tester, 2007)	HEK293 Lipid bilayers	Gain-of-function
FKBP binding dom.	P2328S	CPVT	Decreased FKBP-12.6 binding, PKA-dependent increased RyR2 activity (Lehnart *et al.*, 2004)	HEK293 Lipid bilayers	Gain-of-function
FKBP binding dom.	N2386I	ARVD	Increase caffeine-dependent, sensibility to low cytosolic Ca2+ (Thomas, 2005)	HEK293	Gain-of-function

Location in RyR2	Mutation	Disease	Characterization	System	RyR2 defect
FKBP binding dom	R2474S	CPVT	Enhanced SOICR, increased sensitivity to luminal Ca^{2+} (Jiang D, 2004); (Wehrens *et al.*, 2003); abnormal zipping-unzipping interaction, increase caffeine-dependent, sensibility to cytosolic Ca^{2+} (Yang, 2006); increased frequency of spontaneous Ca^{2+} transients and increased sensitivity to luminal Ca^{2+} mediated by defective interdomain interaction. (Uchinoumi *et al.*, 1998)	HEK293 Lipid bilayers Permeabilized myocytes from rats Knock-in mice	Gain-of-function
FKBP binding dom.	T2504M	ARVD	R176Q/T2504M increase caffeine-dependent sensibility to cytosolic Ca^{2+} (Thomas, 2005); R176Q/T2504M enhance SOICR and increase sensitivity to luminal Ca^{2+} (Jiang D, 2005)	HEK293 Lipid bilayers	Gain-of-function
TM Domain	N4104K	CPVT	PKA and caffeine-dependent increased RyR2 activity (George *et al.*, 2003); abnormal I domain interaction (George CH., 2006); enhanced SOICR (Jiang D, 2005); increased sensitivity to luminal Ca2+ (Jiang D, 2004)	HEK293 Lipid bilayers CHO HL-1	Gain-of-function
TM Domain	Q4201R	CPVT	Ehanced SOICR, increased sensitivity to luminal Ca^{2+} (Jiang D, 2005); decreased FKBP-12.6 binding, PKA-dependent increased RyR2 activity (Lehnart *et al.*, 2004)	HEK293 Lipid bilayers	Gain-of-function
TM Domain	R4497C	CPVT	PKA-dependent increased RyR2 activity (Wehrens *et al.*, 2003); PKA and caffeine-dependent increased RyR2 activity (George *et al.*, 2003); abnormal I domain interaction (Uchinoumi *et al.*, 1998); enhanced SOICR (Jiang D, 2005); increased sensitivity to luminal Ca2+ (Jiang *et al.*, 2004); increased sensitivity to low cytosolic Ca^{2+}, caffeine-dependent increased RyR2 (Jiang *et al.*, 2002); Increased sensitivity to cytosolic Ca^{2+} , triggering activity in ventricular myocytes in presence of high pacing rate and isoproterenol. (Fernandez-Velasco *et al.*, 2009)	HEK-293 Lipid bilayer CHO HL-1 Knock-in ce	Gain-of-function

Location in RyR2	Mutation	Disease	Characterization	System	RyR2 defect
TM Domain	V4653F	CPVT	Decreased FKBP-12.6 binding, PKA-dependent increased RyR2 activity (Lehnart *et al.*, 2004); Increased sensitivity to cytosolic Ca^{2+} (Tester, 2007)	HEK293 Lipid bilayers	gain-of-function
TM Domain	A4860G	IVF	Reduced sensitivity to luminal Ca^{2+}, reduced SOICR activity (Jiang D, 2007)	HEK293 Lipid bilayers	Loss-of-function
C-term	I4867M	CPVT	Enhanced SOICR, increased sensitivity to luminal Ca^{2+} (Jiang D, 2005)	HEK293 Lipid bilayers	Gain-of-function
C-term	N4895D	CPVT	Enhanced SOICR, increased sensitivity to luminal Ca^{2+} (Jiang D, 2004)	HEK293 Lipid bilayers	Gain-of-function

Table 1. RyR2 mutations linked to SCD disease characterized so far. Amino acid mutations are listed in order according to the mutational locus. CPVT=Catecholaminergic Polymorphic Ventricular Tachycardia; ARVD=Arrhythmogenic Right Ventricular Dysplasia; IVF = Idiopathic Ventricular Fibrillation induced by emotion or exercise. TM = Transmembrane domain; SUO = syncope of unknown origin;; SOICR = Store-operated induced- Ca2+ release; HEK293 = Human embrionic kidney cell line; CHO = Chinese hamster ovary cell line; HL-1 = cardiac myocyte cell line.

Because animal's models can contribute to the better understanding of the molecular mechanisms involved in the arrhythmogenic disease, transgenic mice models that harbor some of the most important RyR2 mutations observed in CPVT patients were developed (Uchinoumi *et al.*, ; Cerrone *et al.*, 2005; Kannankeril *et al.*, 2006).

These animals mimic several of the abnormal electrical events observed in CPVT subjects. Indeed, delayed after depolarization (DADs) and triggered activity have been detected in knock-in models of CPVT (Liu *et al.*, 2006). It has been proven by different authors that cardiac myocytes isolated from CPVT models show abnormal diastolic Ca^{2+} release (Ca^{2+} leak) as Ca^{2+} sparks and/or Ca^{2+} waves, which may conduce to arrhythmia by DADs (Uchinoumi *et al.*, ; Kannankeril *et al.*, 2006; Fernandez-Velasco *et al.*, 2009).

One mice model that harbor RyR2 (R2474S) mutation leading to CPVT upon exercise and β-adrenergic stimulation was developed by Lehnart et al., (Lehnart *et al.*, 2008). Cardiomyocytes isolated from R2474S mice exhibited abnormal calcium diastolic leak, calcium waves, APs and inward currents upon isoproterenol treatment. Tonic-clonic seizures were identified in these mice, consistent with the neurological dysfunction including epilepic seizures detected in CPVT patients (Leenhardt *et al.*, 1995; Postma *et al.*, 2005; Lehnart *et al.*, 2008).

Another mechanism by which mutation can alter the calcium handling in CPVT is the disruption of protein-protein interaction. In this context, Wehrens et al., established a direct link between FKBP12.6 and CPVT process (Wehrens *et al.*, 2004). FKBP12.6 (calstabin 2) is an accessory subunit that maintains the RyR2 closed, avoiding calcium leak during diastole. Wehrens et al., proposed that CPVT mutations induce the dissociation of FKBP12.6 from RyR2 upon β-adrenergic stimulation. Therefore, this effect induces a deregulation of the

RyR2 gating, increasing the calcium diastolic release and promoting cardiac arrhythmias by delayed after depolarizations (Marx SO, 2000). Indeed, these authors showed that the presence of different CPVT mutations decreases the affinity of FKBP12.6 binding to RyR, leading to calcium leak (Marx SO, 2000; Wehrens *et al.*, 2003; Lehnart *et al.*, 2008)}. However, these findings have not been confirmed by others groups (Tiso *et al.*, 2002; George *et al.*, 2003; Liu *et al.*, 2006; Xiao *et al.*, 2007; Guo *et al.*, 2010).

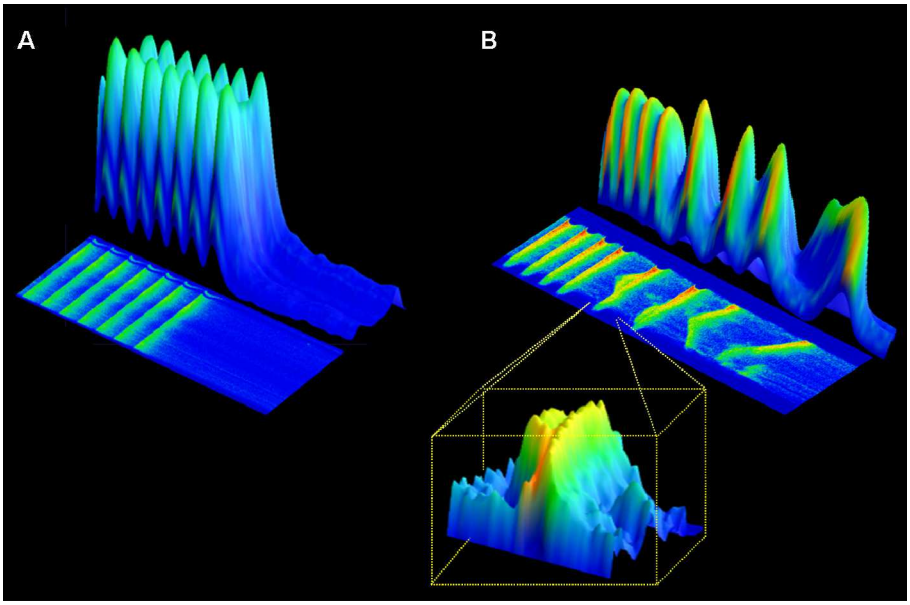

Fig. 2. Triggered activity observed in RyR2R4496C myocytes. 3D line-scan images of ventricular myocytes isolated from (A) a wild type mouse and (B) RyR^{R4496C} mouse during electrical stimulation (4Hz). RyR^{R4496C} cell shows Ca^{2+}-waves that induce consistent with DADs. Finally Matsusaki's group has described altered interdomain RyR2 interactions in CPVT (Ikemoto & Yamamoto, 2000; Tateishi *et al.*, 2009). They proposed that under physiological conditions, N and central terminal domains of RyR2 interact, maintaining the channel closed. CPVT-linked mutations in the N or central domain, causing a disruption of the interaction (domain unzipping), rendering the channel more sensitive to changes in luminal or cytosolic calcium. So, it is reasonable to hypothesize that mutations in these regions of RyR2 could affect the physiological conformational states, resulting in channel dysfunction, as occurs in CPVT (Lobo & Van Petegem, 2009; Tung *et al.*, 2010).

On the other hand, our group using a *knock-in* mice model that express R4496C mutation in the cardiac RyR2 (the equivalent mutation found in CPVT patients, R4497C) demonstrated an enhanced of Ca^{2+} sensitivity of the mutant RyR2 (Fernandez-Velasco *et al.*, 2009). This mice model mimics extraordinarily the clinical manifestations of patients presenting the RyR2R4497C mutation, including the bidirectional VT. RyR2R4496C cardiomyocytes exposed to adrenaline and caffeine developed DADs, suggesting that triggered arrhythmias are elicited by adrenergic activation (Nakajima *et al.*, 1997; Liu *et al.*, 2006). We demonstrated

that untreated RyR2R4496C myocytes have increased spontaneous Ca^{2+} release in diastole during electrical pacing, due to the enhanced Ca^{2+} sensitivity of mutant RyR2; this abnormality is further augmented by exposure to isoproterenol and increasing pacing rates (Figure 2).

4.4 Mutations in calsequestrin 2 linked to CPVT

As previously mentioned, alterations in the control of Ca^{2+} release by changes in luminal calcium can induce serious disruptions in the Ca^{2+} cycling. This is what happens in the recessive form of CPVT associated with mutations in calsequestrin (CASQ2) (Eldar *et al.*, 2003). CASQ2 is a polymer with a low-affinity and high capacity of calcium binding located in the luminal side of SR (Beard *et al.*, 2004; Gyorke & Terentyev, 2008). Although it is documented that CASQ2 interacts with RyR2 via triadin and junctin, and acts as luminal calcium sensor by inhibiting RyR2 function at low luminal calcium concentration (Gyorke *et al.*, 2004; Terentyev *et al.*, 2007), the exact mechanism by which this protein exerts their function is not completely understood.

To date, 12 CASQ2-mutations and 3 non synonymous polymorphisms have been detected in CPVT subjects (http://www.fsm.it/cardmoc/). Although some of these mutations affect the protein synthesis, reducing significantly the CASQ2 expression in the heart, others induce a defective protein expression and alter the ability of SR calcium buffering (Postma *et al.*, 2002; Terentyev *et al.*, 2003; di Barletta *et al.*, 2006; Knollmann *et al.*, 2006; Terentyev *et al.*, 2006). Related to this, different authors have shown in murine cardiomyocytes and in heterologous systems, that mutants of CASQ2 including CASQ2 (L167H), CASQ2 (G112+5X), CASQ2 (R33Q), CASQ2-/- and CASQ2 (D307H) induce a deregulation of SR Ca^{2+} release, leading to arrhythmogenic DADs (Lahat *et al.*, 2001a; Lahat *et al.*, 2001b; Terentyev *et al.*, 2003; di Barletta *et al.*, 2006; Knollmann *et al.*, 2006; Terentyev *et al.*, 2006; Dirksen *et al.*, 2007). These observations are consistent with the ECG pattern observed in CPVT patients (Napolitano & Priori, 2007).

Missense and nonsense CASQ2 mutations have been reported. Regarding missense mutations, CASQ2 (D307H), CASQ2 (R33Q) and CASQ2 (L67H) have been found in CPVT subjects (Terentyev *et al.*, 2006; Kim *et al.*, 2007; Qin *et al.*, 2008; Terentyev *et al.*, 2008). These mutations alter the CASQ2 interaction with RyR2, compromising its ability to store Ca^{2+} in the SR. So far, it has been reported that there are four nonsense mutations that cause the protein to be reduced or deleted (di Barletta *et al.*, 2006). In vivo, CASQ2-/- mice exhibit CPVT with a bidirectional QRS pattern, the classic ECG feature observed in their human disease (Knollmann *et al.*, 2006).

Cellular arrhythmias were detected in cardiomyocytes expressing CASQ2 mutants under β-adrenergic stimulation (Terentyev *et al.*, 2003; di Barletta *et al.*, 2006; Dirksen *et al.*, 2007). These results are consistent with the arrhythmogenic storm elicited by the emotional or physical stress in CPVT patients.

Because there is a correlation between the spontaneous Ca^{2+} release and the DADs, the question is why the spontaneous calcium release occurs. Different approaches address that mutations in CASQ2 compromise the two principal functions described for this protein: as a SR Ca^{2+} storage site and as modulator of RyR2 activity (Kubalova *et al.*, 2005; Terentyev *et al.*, 2006; Terentyev *et al.*, 2008; Knollmann, 2009). Both mechanisms elicited an abnormal

control of RyR2 by luminal Ca^{2+} required to effective termination of SR Ca^{2+} release, promoting the spontaneous Ca^{2+} release during diastole. Studies using transgenic CPVT mice models with CASQ2 mutations confirm that the underlying mechanism of ventricular arrhythmias are DADs caused by spontaneous Ca^{2+} release under adrenergic stress (Mohamed *et al.*, 2007). However, it is important to note that compensatory changes observed in the CASQ2 transgenic mice can alter the junctin and/or triadin function and may affect the manifestation of CPVT under chronic procedures. (Knollmann *et al.*, 2006; Song *et al.*, 2007).

5. Supraventricular arrhythmias in CPVT

CPVT patients often develop supraventricular arrhythmias as resting bradycardia and His-Purkinje block (Sumitomo *et al.*, 2003; Cerrone M, 2007; Sumitomo *et al.*, 2007; Kazemian P, 2011; Sy RW, 2011). Supraventricular arrhythmias (SVAs) are an important issue to underline during following-up patients with CPVT. Although the risk for SCD in CPVT patients is expected to be associated with ventricular arrhythmias, supraventricular abnormalities as sinus node dysfunction, atrioventricular block and supraventricular tachyarrhythmias result in significant increase of morbidity. Moreover, the frequent association of supraventricular arrhythmias in CPVT patients, which has been reported to precede or coexist with ventricular tachycardia, suggests that SVAs may be an important risk factor for SCD in this patient population (Sumitomo *et al.*, 2007; Sy RW, 2011). These SVAs in single point mutations of RyR2 are usually bradycardia, atrial tachycardia, atrial fibrillation and atrioventricular reentry (Sumitomo *et al.*, 2003; Sumitomo *et al.*, 2007; Kazemian P, 2011; Sy RW, 2011). Additionally, Bhuiyan and coworkers found CPVT patients from 2 unlinked families with a deletion of 35 peptides in RyR2 exon-3, who presented also SVAs (sinus bradycardia, sinus block or arrest, atrioventricular block, atrial fibrillation and atrial standstill). Interestingly, these patients also presented left ventricular dysfunction and dilatation, which are rare in CPVT patients.

Additionally, prevention of SVAs is also important in CPVT disease due to potential complications with implantable cardioverter- defibrillators (ICD) therapy. In CPVT, beta-blockers are recommended, together with exercise restraint. Despite beta-blocker therapy, ICD are implanted in patients with previous cardiac arrest, or with recurrent syncope or documented ventricular tachycardia. However, SVAs are not benign arrhythmias in patients with CPVT; they potentially can trigger fast ventricular tachycardias and inappropriate ICD discharges that may lead to fatal ventricular arrhythmias (Pizzale *et al.*, 2008). In the study by Sy and co-workers (Sy RW, 2011), one patient died of refractory ventricular tachycardia/ventricular fibrillation caused by inappropriate ICD shocks of rapidly conducting atrial fibrillation.

The cellular mechanism of sinus bradycardia in CPVT is still unexplored. One possibility is that the function of one or several ion channels that participate in the Ca^{2+} clock mechanism is altered (i.e. NCX, L-type Ca^{2+} channel, etc.) and this abnormality may correspond to a Ca^{2+} and voltage clock uncoupling. It has been reported that the CPVT-linked mutation R4496C presents an increased "SR Ca^{2+} leak" in ventricular myocytes isolated from a transgenic mice (Fernandez-Velasco *et al.*, 2009). A combined genetic and functional approach would be highly required to explore the involvement of RyR mutations on bradycardia and sino-atrial node dysfunction in CPVT disease.

6. Purkinje conduction system

The His-Purkinje system is responsible of the propagation for the action potential to the ventricles. The electrical properties of Purkinje cells are different from those of nodal cells, because they displayed low pacemaker activity and even slower conduction rate. However, Purkinje cells exhibit long action potential duration (APD) and therefore they are prone to EAD and DAD formation (Makarand Deo, 2010). This long APD provides sufficient time for L-type calcium channel reactivation, leading to EAD or DAD formation, which can lead to ectopic beats. Although EADs formation in Purkinje fibers has been quite explored (Fedida D, 2006), little is known concerning the development of DADs in these cardiac conducting fibers (GR., 1980; Gough WB, 1989). It has been recently described that DADs are associated to the development of CPVT disease (Liu *et al*., 2006; Fernandez-Velasco *et al*., 2009), but the mechanism of how DADs induce bidirectional VT is unknown. To fulfill this question, Cerrone and co-workers performed whole-heart optical mapping in heart isolated from knock-in mice carrying the R4496C mutation, and they found that bidirectional VT was caused by two foci in the distal His-Purkinje system, one in the right ventricle and the other in the left ventricle, activating the ventricles alternatively (Cerrone M, 2007). Polymorphic VT was initially multifocal but eventually became reentrant and degenerated into ventricular fibrillation. Moreover, chemical ablation of the right ventricular His-Purkinje system with Lugol solution converted bidirectional VT to monomorphic VT in mice. The same group further demonstrated in an additional report that Purkinje cells are more sensitive to the R4496C RyR2 mutation than ventricular myocytes, which strongly supports the idea that Purkinje cells are responsible of the arrhythmia in CPVT (Herron TJ, 2010). The latter result was further explored by other work, which has also tested that Flecainide reduce spontaneous Ca^{2+} release in Purkinje cells (Kang G, 2010).

6.1 Treatment of CPVT

Although β-adrenergic blockers is the most common treatment chosen for CPVT patients, they are incompletely effective with up to 30% of subjects requiring implantable cardioverter-defibrilators (ICDs) (Priori *et al*., 2002). In severe cases of CPVT, the nondihydropyridine Ca^{2+} channels blockers may also be effective (Swan *et al*., 2005; Rosso *et al*., 2007).

Dantrolene, a drug used to prevent malignant hyperthermia in patients with mutations in RyR1 who have been exposed to volatile anaesthetics, has been proposed to have therapeutic potential in heart disease by causing "rezipping" of the amino and central domains of RyR2 (Kobayashi *et al*., 2009; Kobayashi *et al*., 2010).

More recently, a protective effect of flecainide was proposed for CPVT treatment, thus this drug is able to block Ca^{2+} leak from RyR2 in CASQ2 deficient mice (Watanabe *et al*., 2009).

Finally, the use of drugs (JTV519) with selective action on FKBP 12.6 is still remaining in discrepancy, because diverging data to this respect were obtained.

7. Arrhythmogenic right ventricular dysplasia

Arrhythmogenic right ventricular dysplasia (ARVD) is a genetic form of cardiomyopathy that by contrast to CPVT, primarily affects the right ventricle (RV) and is characterized by

the abnormal replacement of myocytes by adipose and fibrous tissue (Basso *et al.*, 2009). The estimated prevalence of ARVD in general population ranges from 1 in 2000 to 1 in 5000 (Corrado *et al.*, 1997) and is more frequent in men than in women, being a major cause of sudden death in the young and in athletes.

ARVD was initially believed to be a developmental defect of the RV myocardium, leading to the original designation of dysplasia (Basso *et al.*, 1996). The diagnostic of ARVD patients including MRI, echocardiography, electrocardiography and right ventricle biopsy (McKenna *et al.*, 1994). ARVD is characterized by functional abnormalities of the right ventricle, with abnormal depolarization/repolarization, leading to syncope, ventricular arrhythmias and sudden death (Rossi *et al.*, 1982). Interestingly, in a high percent of patients left ventricular dysfunction was found (Corrado *et al.*, 1997).The most typical clinical presentation of ARVD is symptomatic ventricular arrhythmias of right ventricular origin, usually triggered by effort.

ARVD can be inherited as an autosomal dominant disease with reduced penetrance and variable expression, although autosomal recessive forms also have been detected (Rampazzo *et al.*, 2002). Mutations in genes encoding for different molecules have been linked to ARVD. To this regard, mutations in adhesion proteins (plakoglobin, desmoplakin, plakophilin-2 and desmoglein-2), in cytokines (Transforming grow factor beta 3), in transmembrane protein 43 and in RyR2 have been detected in ARVD subjects (Tiso *et al.*, 2001; Rampazzo *et al.*, 2002; Gerull *et al.*, 2004; Beffagna *et al.*, 2005; Pilichou *et al.*, 2006; Merner *et al.*, 2008).

Regarding RyR2 mutations, ARVD patients with mutations in RyR2 tend to have mild ARVD symptoms and are classified as ARVD2. The R176Q mutation has been associated with the ARVD disease and also carries out a second mutation of T2504M (Tiso *et al.*, 2001). Both mutations induced the increased RyR activity in vitro (Thomas *et al.*, 2004).

The mice model that harbors R176Q mutation allowed for the better understanding of this arrhythmogenic disease. Hearts from R176Q heterozygous mice were structurally normal, but under β-adrenergic stimulation, myocytes elicited oscillatory Ca^{2+} signals, leading to mice VT (Kannankeril *et al.*, 2006).

7.1 Treatment of ARVD

There is not a curative treatment, instead, the aim is to detect patients with high risk and prevent complications.

The four therapeutic options are pharmacological agents (first choice), catheter ablation (if the patient is refractory to drug treatment or the disease is localized), implantable cardioverter-defibrillators (in refractory subject at risk for sudden death) and surgery as the last option (ventriculotomy and disconnection of the RV free wall) or cardiac transplantation (if severe terminal heart failure) (McKenna *et al.*, 1994).

As we mentioned, the first option for ARVD patients is the pharmacological treatment, including ACEI, anticoagulants, antiarrhythmic agents as sotalol, verapamil, beta-blockers, amiodarone and flecainide.

In conclusion, genetic analysis is essential in both CPVT and ARVD patients, because if a pathogenic mutation is identified, a pre-symptomatic diagnosis of the disease among family

members might be provided and also the development of the disease can be monitored to assess the risk of transmitting them offspring.

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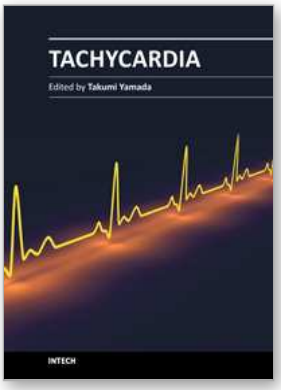

Tachycardia

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Heart rates are normally controlled by a natural pacemaker, the sinus node, and normal heart rhythm is called sinus rhythm. Tachycardia is defined as a faster heart rhythm than normal sinus rhythm. Tachycardias can cause symptoms such as palpitations, chest pain, shortness of breath and fatigue, which reduce the quality of life. Fast tachycardias can cause hemodynamic collapse and sudden cardiac death. The causes, mechanisms, and origins of tachycardias are various. The diagnosis of tachycardias is made by electrocardiograms and electrophysiological testing. Tachycardias can be managed and treated by pharmacological and non-pharmacological approaches. This book covers these concerns from basic and clinical points of view and will lead to a further understanding and improvement in the clinical outcomes of tachycardias.

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