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Review

Calcium Release Deficiency Syndrome (CRDS): Rethinking "Atypical" Catecholaminergic Polymorphic Ventricular Tachycardia

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Abstract: Since the first description of catecholaminergic polymorphic ventricular tachycardia (CPVT) in the 1970s, new insights have progressively unraveled the understanding of this inherited arrhythmia syndrome. The identification of new distinct clinical entities related to *RYR2*, the gene encoding the cardiac ryanodine receptor, has allowed significant refinement in the diagnosis of previously labeled "atypical" CPVT cases. Among *RYR2*-ryanodinopathies, the characterization of calcium release deficiency syndrome (CRDS) is still in its infancy and represents a diagnostic challenge due to the need for functional studies which may confirm the loss-of-function nature of the *RYR2* variant. The present review summarizes current evidence on CRDS. First, by providing an overview on *RYR2* structure and function, we will elucidate the different pathophysiological underpinnings of CRDS and CPVT. Second, by retrieving in detail reported CRDS variants and their clinical phenotypes, we will provide, if any, genetic and clinical red flags that should raise suspicion for CRDS in daily clinical practice. Finally, we will discuss available therapies to provide clinicians with practical therapeutic options for CRDS management.

Keywords: calcium release deficiency syndrome; catecholaminergic polymorphic ventricular tachycardia; *RYR2*; ryanodinopathies; clinical genetics; loss-of-function variants

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1. Introduction

Since the first cases described in the 1970s by Reid et al. [1] and Coumel et al. [2], the clinical spectrum of catecholaminergic polymorphic ventricular tachycardia (CPVT) has significantly evolved. Leenhardt et al. [3] initially stated in 1995 that CPVT arrhythmic features were "reproducibly induced by any form of increasing adrenergic stimulation". Later, in 2002, Priori et al. [4] reported that 13% of their CPVT probands presented with unexplained cardiac arrest, without any inducible arrhythmia at the exercise stress test. Since then, subsets of CPVT patients were labeled as suffering from catecholaminergic idiopathic ventricular fibrillation, or as "atypical" CPVT. More recently, however, with increasing insights into the causative role of *RYR2* (the gene encoding the cardiac ryanodine receptor) in CPVT, the identification of new distinct clinical entities have allowed significant refinement of the diagnosis and elucidation of the phenotypes of "atypical" CPVT cases. The term "ryanodinopathies" [5] was then introduced encompassing, besides typical CPVT, exon 3 deletion syndrome (E3DS) and calcium release deficiency syndrome (CRDS). Both first described in 2007 [6,7], E3DS is characterized by a pleiotropic expression, potentially combining a tachycardia-bradycardia phenotype to mitral valve prolapse and to dilated cardiomyopathy, with or without left ventricular non-compaction [5]. The diagnosis of E3DS is confirmed by the skipping of exon 3 (c.161 to c.272), leading to a 35 amino acid in-frame deletion at the N-terminal domain of the cardiac ryanodine receptor (RYR2) [5].

Conversely, the CRDS phenotype may remain undetectable at cardiac work-up and diagnostic confirmation is still hampered by the need for in vitro studies. Different from CPVT, which is caused by gain-of-function (GOF) *RYR2* variants, the CRDS hallmark is the presence of loss-of function (LOF) *RYR2* variants. Once a novel *RYR2* variant is identified, only functional studies may allow CRDS diagnosis by providing critical evidence to elucidate the molecular mechanism underlying the variant. Since functional studies are not yet available in daily practice, this makes most CRDS cases unrecognized or misdiagnosed.

Our review will summarize current evidence on CRDS. First, by providing an overview on *RYR2* structure and function, we will elucidate the different pathophysiological underpinnings between CRDS and CPVT. Second, by retrieving in detail reported CRDS variants and their clinical phenotypes, we will provide, if any, genetic and clinical red flags that should raise suspicion for CRDS into daily clinical practice. Finally, we will discuss available therapies to provide clinicians with practical therapeutic options for CRDS management.

2. RYR Genes and Proteins

The discovery of the ryanodine receptor dates back to the 18th century, when a new plant from South America was first described by the Danish botanist Martin Vahl, who named the plant *Ryana Speciosa* as a tribute to his friend John Ryan, who sent him the sample [8]. In 1948, the natural insecticide isolated from *Ryana Speciosa* was called "ryanodine" [8,9] which subsequently proved to interact with channels of the sarcoplasmic reticulum (SR) involved in calcium release [8,10]. The ryanodine receptor was finally discovered and purified [8,11] thanks to ryanodine radiolabeling and the three main complementary DNAs (cDNAs) were progressively cloned in mammalian species [8,12–16] in the early 1990s.

Human ryanodine receptors are encoded by three different genes (*RYR1*, *RYR2*, and *RYR3*) located on different chromosomes (19q13.2, 1q43, and 15q13.3-q14). Despite being ubiquitous, RYR1 and RYR2 are preferentially expressed in skeletal and cardiac muscles, respectively, while RYR3 is widely expressed, even if originally identified on brain samples. The three isoforms share 66% of their amino acid sequence and differences are clustered in three main divergent regions, accounting for 11% of the overall sequence and probably explaining isoform-specific clues [8,17].

Expressed early during embryogenesis [18], RYRs are intracellular tetrameric ion channels that play a pivotal role in excitation-contraction coupling, the process which converts the electrical signal—represented by plasmatic membrane depolarization—into the mechanical output of contraction by means of calcium signaling [8,19].

3. The Cardiac RYR2 Isoform

The *RYR2* gene encompasses 105 exons coding for a large 4967 amino acid protein. The cardiac ryanodine receptor derives from the assembly of four RYR2 polypeptides in a large homotetrameric ion channel of about 2.2 MDa, located within the SR membrane. The structure consists of an N-terminal mushroom-like cytoplasmic shell, an activation domain, and a transmembrane pore [18,19]. The activity of RYR2 is subject to complex regulatory mechanisms involving post-translational modifications, (i.e., phosphorylation, nitrosylation, etc.), and multiple functional ligands (calcium ions (Ca^{2+}), calmodulin, calstabin, etc.). These latter, by interacting at specific binding sites, can steer the channel towards specific functional states [18,19].

Following sarcolemma and T tubule depolarization in normal cardiomyocytes, the opening of the L-type voltage-gated Ca^{2+} channels allows the cytoplasmatic influx of Ca^{2+} ions from the extracellular space. Through the interaction with specific high-affinity sites, Ca^{2+} ions activate RYR2 and trigger the "calcium-induced calcium release" (CICR) process, since the opening of RYR2 gating promotes the supplemental release of Ca^{2+} stored in the SR [17]. Muscular contraction can take place thereafter thanks to the interaction between Ca^{2+} ions and troponin C, the Ca^{2+}-sensing protein responsible for myofilament

contractions. Once relaxation occurs, the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase 2a (SERCA2a) pumps back part of the cytosolic Ca^{2+} content into the SR while the Na^{+}-Ca^{2+} exchanger (NCX) channel extrudes another part into the extracellular space. Figure 1.

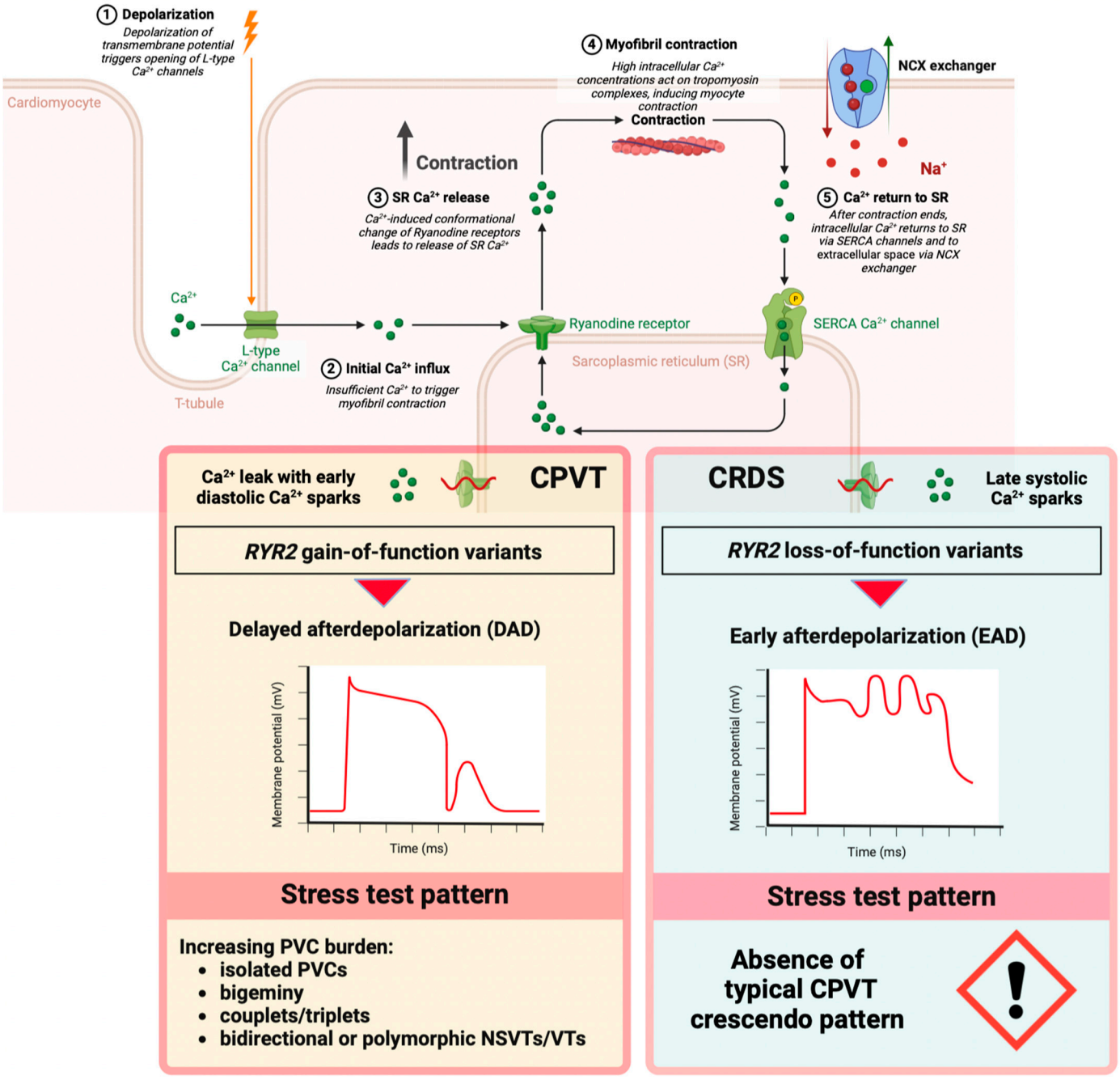

Figure 1. The calcium interaction network and pathophysiological/clinical differences between CPVT and CRDS. (①–②) Following sarcolemma and T tubule depolarization, opening of the L-type voltage-gated Ca^{2+} channels permits cytoplasmatic influx of Ca^{2+} ions from the extracellular space. ③ Ca^{2+} ions activate RYR2 and trigger the "calcium-induced calcium release" (CICR) process, with supplemental release of Ca^{2+} stored in the sarcoplasmic reticulum (SR). ④ Muscular contraction can take place. ⑤ Once relaxation occurs, the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase 2a (SERCA2a) pumps part of the cytosolic Ca^{2+} content back into the SR while the Na^{+}- Ca^{2+} exchanger (NCX) channel extrudes another part into the extracellular space. PVCs: premature ventricular contractions; NSVTs: non-sustained ventricular tachycardias; VTs: ventricular tachycardias.

Of note, the relation between Ca^{2+} concentration and Ca^{2+} conductance displays a bell shape: increasing Ca^{2+} concentration progressively augments the probability of channel opening through the interaction with its high-affinity Ca^{2+}-binding sites. When Ca^{2+} concentration exceeds a given threshold, the probability of channel closing increases, through the interaction between Ca^{2+} ions and RYR2 low-affinity Ca^{2+}-binding sites.

4. *RYR2*-Related Disease Mechanisms

RYR2 pathogenic variants are responsible for about 80% of all CPVT cases, accounting for the so-called CPVT type 1 [5,20–22]. Such pathogenic variants are mainly missense and lead to RYR2 gain-of-function (GOF) by disrupting channel stability, following the variant-induced increased sensitivity to luminal Ca^{2+}. Through a mechanism of store overload-induced Ca^{2+} release (SOICR), the threshold for spontaneous Ca^{2+} release from the SR is decreased, leading to cytoplasmatic Ca^{2+} leakage from the SR during diastole [23,24]. In the attempt to extrude Ca^{2+} ions released, the NCX embedded in the plasmatic membrane generates a depolarizing transient inward current (I_{ti}), hence promoting delayed afterdepolarizations (DADs) [23,25]. If sufficiently large, this depolarizing inward current may ignite the electrical process of arrhythmogenesis through action potential activation, leading to triggered activity [25]. Of note, few other genes have been causally associated to less frequent subtypes of CPVT, which have been described in detail elsewhere [21,22].

Conversely, CRDS patients carry loss-of-function (LOF) *RYR2* pathogenic variants. As demonstrated by Sun et al. [26] on knock-in mouse models expressing *RYR2* CRDS variants, these latter translated into a loss of channel sensitivity to Ca^{2+} luminal content and into depressed SOICR. Such reduced channel activity and the subsequent impairment of Ca^{2+} autoregulation trigger a temporal and spatial desynchronization of SR Ca^{2+} release, leading to Ca^{2+} alternans and hence to electromechanical alternans [7,23]. Moreover, LOF *RYR2* variants prompt a major electrophysiological remodeling which affects the Ca^{2+} interaction network. Due to RYR2 impaired activity, the decreased SR Ca^{2+} release induces both an increased storage of Ca^{2+} into the SR and a compensatory enhanced cytosolic Ca^{2+} inflow/outflow through the L-type Ca^{2+} channels and NCX, respectively [23]. An increase of the transient outward current (I_{to}) has been similarly reported [23,26]. Such alterations represent the electrical substrate underlying the prolonged cardiac action potential and the increased likelihood of early afterdepolarization (EAD) [23,26], as seen in Figure 1.

The work by Sun et al. [26] elegantly explored the mechanisms underpinning arrhythmogenesis in mice cardiomyocytes carrying CRDS variants, by analyzing the intracellular Ca^{2+} dynamic. They demonstrated the specific propensity of CRDS hearts to develop ventricular arrhythmias following Ca^{2+} transient irregularity triggered by long-burst, long-pause, and short-coupled extra-stimulus (LBLPS) sequences [26]. During the tachycardia burst phase, an increase in cytosolic Ca^{2+} content results from the imbalance between Ca^{2+} inflow (through the L-type Ca^{2+} channels) and its cytosolic removal (through NCX and SERCA2a). The following long pause is characterized by the sequestration of accumulated cytosolic Ca^{2+} into an already high-stored SR (through SERCA2a). In this context and despite its constitutive impairment, the CRDS RYR2 channel conveys an aberrant Ca^{2+} release from an overfilled SR at the following short-coupled extra-stimulus, thereby enhancing NCX activity and EAD propensity [23].

In summary, while in CPVT the spontaneous diastolic SR Ca^{2+} release accounts for DAD-mediated ventricular arrhythmias, CRDS is characterized by systolic Ca^{2+} dysregulation acting both as the trigger and the vulnerable substrate for EAD-driven arrhythmias [26].

5. CRDS Genotype: Are There Any Genetic Red Flags?

Up to date, thirty-three *RYR2* variants have been reported in literature with proven evidence for the LOF effect at in vitro functional studies. In 2007, Jiang et al. [7] described the first LOF *RYR2* missense variant (p.A4860G) in a 7-year-old patient, previously enrolled in the study by Priori et al. [4] and labeled as suffering from atypical CPVT. Only in 2016, a second LOF *RYR2* variant (p.S4938F) was identified by Fujii et al. [27] in a proband

presenting with short-coupled torsades de pointes, raising the compelling suspicion of a "novel arrhythmia syndrome" [28] distinct from CPVT. Since then, few anecdotical cases or small case series were reported [29–32] until 2021, when two major works were published. The first, by Sun et al. [26], provided clinical, genetic, and functional insights on six *RYR2* LOF mutations (p.Q3774L, p.I3995V, p.D4112N, p.T41961I, p.D4646A, and p.Q4879H) identified in six families, demonstrating their causative role in CRDS. Moreover, the authors first identified the LBLPS stimulation protocol as an effective and specific diagnostic tool for CRDS. The second, a multicenter study by Roston et al. [33], described six supplemental *RYR2* LOF mutations (p.E4451del, p.F4499C, p.V4606E, p.R4608Q, p.R4608W, and p.Q2275H) among 19 patients, increasing the number of reported CRDS cases from fewer than 10 to approximately 30 in the literature [33]. Table 1.

Hitherto, thirty-one out of thirty-three reported mutations are heterozygous missense variants due to single nucleotide variations. One case of heterozygous single amino acid deletion (p.E4451del) was also described [33]. Interestingly, the majority of CRDS variants are located in the C-terminus (amino acids 3778-4959) of *RYR2*, suggesting a potential link between variant location in the transmembrane pore and a LOF effect. However, we do know that some well-recognised CPVT variant hotspots (hotspot 3 and 4) involve the *RYR2* C-terminus as well. For this reason, the within-gene variant topography does not yet represent a reliable marker to distinguish between CRDS and CPVT [23], as seen in Figure 2.

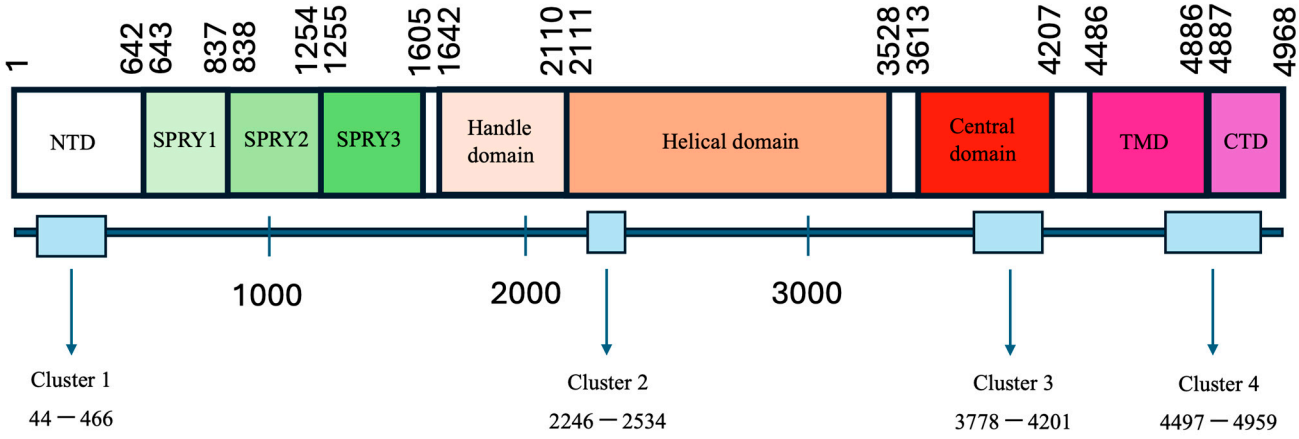

Figure 2. Linear structure of RYR2 protein. Aminoacidic linear structure of RYR2 and major functional domains. Blue boxes identify four known pathogenic variant hotspots.

Of note, all CRDS variants identified so far were autosomal dominantly inherited except for one. In the Amish community, Tester et al. [34] identified a copy number variation (CNV) consisting of a homozygous tandem duplication of 344,085 base pairs involving the 5′ untranslated region of the promoter and exons 1 through 4 of *RYR2* [34,35].

The molecular characterization in a model of induced pluripotent stem cell cardiomyocytes (iPSC-CM) generated from CNV carriers pointed out that this homozygous CNV led to a >50% decrease in *RYR2* transcript and protein expression [34,35]. The resulting haploinsufficiency hence determined an impairment in Ca^{2+} handling and in the CICR process, which become insensitive to caffeine and catecholamines [34,35]. Despite its different transmission pattern, the CNV described by Tester et al. was associated with a highly penetrant and particularly malignant phenotype characterized by exercise-associated sudden cardiac death in the young, intermittent prolonged QT intervals or prominent U-waves, and normal cardiac work-up (i.e., exercise stress test, epinephrine challenge, and 24-h Holter monitor) [23,34,35].

Table 1. Published *RYR2* variants with proven LOF effect.

Nucleotide Change	Amino Acid Change	RYR2 Hotspot	Phenotype	N° of Carriers Among Relatives	References
c.14579C>G	p.A4860G	4	SCD	• 1 asymptomatic • 1 obligate carrier	[7]
c.14813C>T	p.S4938F	4	Sc-TdP	• 1 asymptomatic • 1 obligate carrier	[24]
c.14565T>G	p.I4855M	4	SCA, SUD, LVNC, QT prolongation	• 1 symptomatic • 1 obligate carrier	[26]
c.11321A>T	p.Q3774L	NH	SCA	• 3 asymptomatic	[23]
c.11983A>G	p.I3995V	3	SCD/SCA/SUD	• 7 carriers (3 asymptomatic)	
c.12334G>A	p.D4112N	3	SCD/SCA	• 6 carriers (2 asymptomatic)	
c.12587C>T	p.T41961I	3	SCD/SCA	• 2 symptomatic	
c.13937A>C	p.D4646A	4	SCD/SCA	• 6 carriers (2 asymptomatic)	
c.14637A>C	p.Q4879H	4	SCA	• No carriers	
c.1378A>G/c.6224T>C	p.K4594R/p.I2075T	4 NH	SCD/SCA	• 7 carriers	[36]
c.1709G>A	p.G570D	NH	SCD/SCA	• Unknown	[29]
c.12440G>A	p.R4147K	4	SCA	• 4 asymptomatic	
c.12608C>T	p.A4203V	NH	SCA	• Unknown	
c.12326T>G	p.M4109R	3	SCD/SCA	• 6	
c.12611C>T	p.A4204V	NH	SCA	• Unknown	
c.11773C>G	p. Q3925E	3	SCD	• Unknown	
c.12436G>A	p.E4146K	3	SCD	• Unknown	[28]
c. 14803G>A	p.G4935R	4	SCD	• Unknown	
c.11321A>T	p.Q2275H	2	SCA	• Unknown	[30]
c.11983A>G	p.E4415del	NH	SUD	• 2 asymptomatic	
c.12334G>A	p.F4499C	4	Syncope, AA	• 1 symptomatic	
c.12587C>T	p.V4606E	4	Syncope, AA, DC	• Unknown	
c.13937A>C	p.R4608Q	4	SUD	• 10 carriers (8 asymptomatic)	
c.14637A>C	p.R4608W	4	SCA	• Unknown	

c.9872A>T	p.D3291V	NH	SCD	• 3 families:
				  • Family 1: 39 carriers
				  • Family 2: 6 carriers
				  • Family 3: unknown
					[27]

c.12424G>A	p.A4142T	3	SCD/SCA	• 17 carriers (13 asymptomatic)	[34]
c.13780A>C	p.K4594Q	4	Long QT	• Unknown	[33]
c. 12502T>C	p.S4168P	3	Long QT, bradycardia	• 1 carrier	
c.12438G>T	p.E4146D	3	Long QT, SCA	• Unknown	
c.11321A>T	p.E1127G	NH	SCA	• 1 asymptomatic	[37]
c.11983A>G	p.A3442E	NH	Syncope	• Unknown	
c.12334G>A	p.I3476T	NH	Syncope	• 1 asymptomatic	
Tandem dup 5′UTR/promoter region, exon 1–4	p.?	NA	SCD/SCA	• 14 symptomatic (homozygous and heterozygous)	[31]

SCD: sudden cardiac death; SCA: sudden cardiac arrest; sc-TdP: short-coupled torsades de pointes; SUD: sudden unexplained death; LVNC: left ventricular non-compaction; AA: atrial arrhythmia; DC: dilated cardiomyopathy; NH: not in hotspot; NA: not applicable.

6. CRDS Phenotypes: Are There Any Clinical Red Flags?

Although also reported in childhood and preadolescence, manifestation onset most commonly occurs in early adulthood [5,33], and includes sudden cardiac death/arrest or syncope. According to the recent work by Hirose et al. [36], two major phenotypes may be associated with *RYR2* LOF variants: ventricular fibrillation and short-coupled torsades de pointes in case of variants which moderately impair RYR2 activity, vs. QT prolongation and bradycardia, without lethal arrhythmias, in case of variants which severely suppress RYR2 activity. In the first case, arrhythmogenicity is likely the result of the electrophysiological remodeling prompted by the moderate impairment of RYR2 activity, finally leading to prolonged cardiac action potential and increased likelihood of EAD-driven arrhythmias. Conversely, when RYR2 activity is too severely suppressed, no random Ca^{2+} release seems

to occur after an increased storage of Ca^{2+} into the SR and no afterdepolarizations arise. The reduced amplitude of the Ca^{2+} transient may only induce the suppression of the Ca^{2+} dependent inactivation of L-type Ca^{2+} channels, with subsequent QT prolongation. Moreover, since RYR2 impacts on the Ca^{2+} clock-regulating pacemaker activity in sinoatrial nodal cells, bradycardia may result from the variant-induced severe impairment of RYR2 activity [23,36].

In the largest CRDS cohort reported so far, adrenergic stimulation (exertion/emotion) triggers only 50% of the arrhythmic events [5,33] and breakthrough events despite medical therapy were less frequently reported (19% over an 8-year follow-up [23,33]) than in CPVT. In addition, atrial arrhythmias have been described in 12% of the patients [23] and cardiac structural abnormalities (i.e., non-compaction and dilated cardiomyopathy) have been reported in association with two CRDS variants (p.I4855M [29] and p.V4606E [33]). Since the molecular characterization of the p.V4606E [33] variant pointed out a complete suppression of the channel function, a relation between the extent of the LOF effect and the degree of preservation of myocardial structure/function may be plausible. For this reason, although further insights are still needed on this topic, it could be advisable to perform serial echocardiography among CRDS patients to screen for potential cardiac structural abnormalities [23].

In the study by Roston et al. [33], the exercise stress test was negative in 67% of CDRS patients. Conversely, the remaining 33% presented with isolated ventricular ectopy which was inconsistent with the CPVT phenotype. In these cases, premature ventricular contractions (PVCs) appeared essentially monomorphic, isolated, or non-sustained, without the typical CPVT crescendo pattern with increasing heart rate, and mostly occur during recovery [23]. To avoid CRDS misdiagnosis in these cases, it is advocated to perform a burst exercise stress test to unmask latent CPVT [23].

Finally, as previously described, Sun et al. [26] first identified in 2021 a specific protocol of programmed ventricular stimulation as an effective diagnostic tool for CRDS. They demonstrated the propensity of CRDS mice hearts to develop ventricular arrhythmias following Ca^{2+} transient irregularity triggered by LBLPS sequences. These latter include a long 20-beat burst at 60 ms cycle length followed by a long-coupled ventricular extrastimulus at 122 ms and a short-coupled extra-stimulus, which was progressively reduced from 78 to 18 ms in 4 ms steps [23,26]. Such results were indirectly confirmed by the LBLPS-like pattern recorded before ventricular arrhythmia onset on the devices implanted in CRDS patients [23]. In addition, the protocol was initially tested in two CRDS patients, triggering polymorphic ventricular arrhythmias in both of them [26]. In human subjects, the protocol consisted of a long cycle ("pause") of 800 ms, or 50 ms shorter than the sinus cycle length. Then, the first extra-stimulus (S2) was delivered, followed by a second extra-stimulus at 400 ms (S3) and further decremented by 20 ms intervals down to refractoriness, or 180 ms, whichever occurred first [26,37]. The test was considered positive in case of induced non-sustained ventricular tachycardia or sustained ventricular arrhythmias. In the study by Ormerod et al. [37] the LBLPS protocol was performed in nine additional CRDS patients, inducing monomorphic non-sustained ventricular tachycardia or sustained ventricular arrhythmias requiring cardioversion in 78% of them. Interestingly, the authors also demonstrated a correlation between the severity of arrhythmias induced in the electrophysiological study and the severity of patient's clinical phenotype, providing the first evidence for both a diagnostic and a potential prognostic role for the LBLPS protocol in CRDS. However, due to the invasive nature of the tool and to the detrimental need to induce ventricular arrhythmias for a positive test [38], a very recent work tested a new bedside maneuver to diagnose CRDS. This international multicenter case-control study evaluated the change in T-wave amplitude on the first sinus beat observed after a spontaneous or pacing-mediated (for at least 10 beats at $\geq$150 beats/min) tachycardia followed by a subsequent pause. The authors demonstrated that CRDS patients and mouse models presented with a distinctive electrocardiographic signature represented by a marked increase in QT interval and T-wave amplitude [38]. This particular pattern likely reflects the large calcium

transient occurring after the brief episode of ventricular tachycardia followed by a pause in CRDS patients, providing an important clue to distinguish them from controls (individuals with supraventricular tachycardia, survivors of unexplained cardiac arrest and genetically confirmed CPVT patients) [38].

7. CRDS Treatment Strategy

From the pre-clinical data available so far, betablockers and Flecainide seem to represent valuable candidate medications in CRDS [23]. In a model of iPSC-CMs generated from CRDS carriers, Tester et al. [35] demonstrated that Nadolol and Propranolol effectively reduced the erratic activity below the arrhythmic level of the control iPSC-CMs. Similarly, Flecainide reduced the erratic activity by 2.4-fold, but did not exert a complete rescue of the CRDS arrhythmic phenotype, which remained significantly more severe than that observed in the control iPSC-CMs. In addition, Sun et al. [26] speculated that the simultaneous target of the inward and outward currents disrupted by the electrical remodeling induced by the RYR2 variant may represent a rational therapeutic option. Indeed, both Quinidine and Flecainide (I_{to}, I_{CaL}, and I_{Na} inhibitors) were able to suppress LBLPS-induced ventricular arrhythmias in CRDS model hearts [26].

Focusing on few available clinical data, Flecainide administration completely abolished LBLPS-induced arrhythmias in 89% of CRDS patients [37]. Conversely, an increase in arrhythmia inducibility was observed in 44% of patients when combining Metoprolol and Flecainide [37]. Due to this potential detrimental effect, further clinical studies are needed to assess the safety profile of a dual therapy associating betablockers to Flecainide. On the other hand, when analyzing the largest CRDS cohort reported so far, betablockers were introduced in 94% of patients, among whom 75% were treated with Bisoprolol. If nonadherence to betablockers was responsible for 55% of life-threatening events recorded during a median 8-year follow-up, at least one breakthrough event occurred in 19% of patients, despite betablockers. Conversely, the study included only one patient treated with Flecainide, precluding any potential conclusion about its clinical efficacy.

Finally, the absence of designed clinical studies supporting clear evidence for a specific therapy and the occurrence of documented breakthrough events despite medical therapy may support the use of ICD for primary prevention in CRDS. However, no solid evidence exists on this subject, which still represents an important gap in knowledge and a major clinical challenge, especially when facing young asymptomatic relatives who carry familial CRDS variants.

8. Conclusions

CRDS represents a novel cardiac ryanodinopathy caused by LOF *RYR2* pathogenic variants. These latter are responsible for a major electrophysiological remodeling which, by affecting Ca^{2+} interaction network, results in a prolonged cardiac action potential and an increased likelihood of EAD-driven arrhythmias. Up to now, no distinctive CRDS genetic red flags have been identified, although CRDS mutations are predominantly autosomal dominant missense variants located in the C-terminus of *RYR2*. From a clinical perspective, two major phenotypes appear to be associated with *RYR2* LOF variants: ventricular fibrillation and short-coupled torsades de pointes in case of variants which moderately impair RYR2 activity, vs. QT prolongation and bradycardia, without lethal arrhythmias, in case of variants which severely suppress RYR2 activity. Based on literature data, CRDS clinical red flags might include: (1) pleiotropic phenotypes including ventricular fibrillation, short-coupled torsade de pointes, prolonged QT and bradycardia; (2) absence of polymorphic and/or bidirectional complex ventricular tachyarrhythmias at exercise stress test; (3) positive response to the LBLPS protocol defined by the induction $\geq$4 ventricular ectopic beats after pacing, (4) marked increase in QT interval and T-wave amplitude after brief ventricular tachycardia and pause. Despite interesting pre-clinical findings, proof-of-concept trials on CRDS therapeutic options are still lacking, even if betablockers or flecainide (but not their combination) seem to represent valuable candidate medications.

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