RYR2-ryanodinopathies: from calcium overload to calcium deficiency

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Abstract

The sarcoplasmatic reticulum (SR) cardiac ryanodine receptor/calcium release channel RyR2 is an essential regulator of cardiac excitation–contraction coupling and intracellular calcium homeostasis. Mutations of the RYR2 are the cause of rare, potentially lethal inherited arrhythmia disorders. Catecholaminergic polymorphic ventricular tachycardia (CPVT) was first described more than 20 years ago and is the most common and most extensively studied cardiac ryanodinopathy. Over time, other distinct inherited arrhythmia syndromes have been related to abnormal RyR2 function. In addition to CPVT, there are at least two other distinct RYR2-ryanodinopathies that differ mechanistically and phenotypically from CPVT: RYR2 exon-3 deletion syndrome and the recently identified calcium release deficiency syndrome (CRDS). The pathophysiology of the different cardiac ryanodinopathies is characterized by complex mechanisms resulting in excessive spontaneous SR calcium release or SR calcium release deficiency. While the vast majority of CPVT cases are related to gain-of-function variants of the RyR2 protein, the recently identified CRDS is linked to RyR2 loss-of-function variants. The increasing number of these cardiac 'ryanodinopathies' reflects the complexity of RYR2-related cardiogenetic disorders and represents an ongoing challenge for clinicians.

This state-of-the-art review summarizes our contemporary understanding of RYR2-related inherited arrhythmia disorders and provides a systematic and comprehensive description of the distinct cardiac ryanodinopathies discussing clinical aspects and molecular insights. Accurate identification of the underlying type of cardiac ryanodinopathy is essential for the clinical management of affected patients and their families.
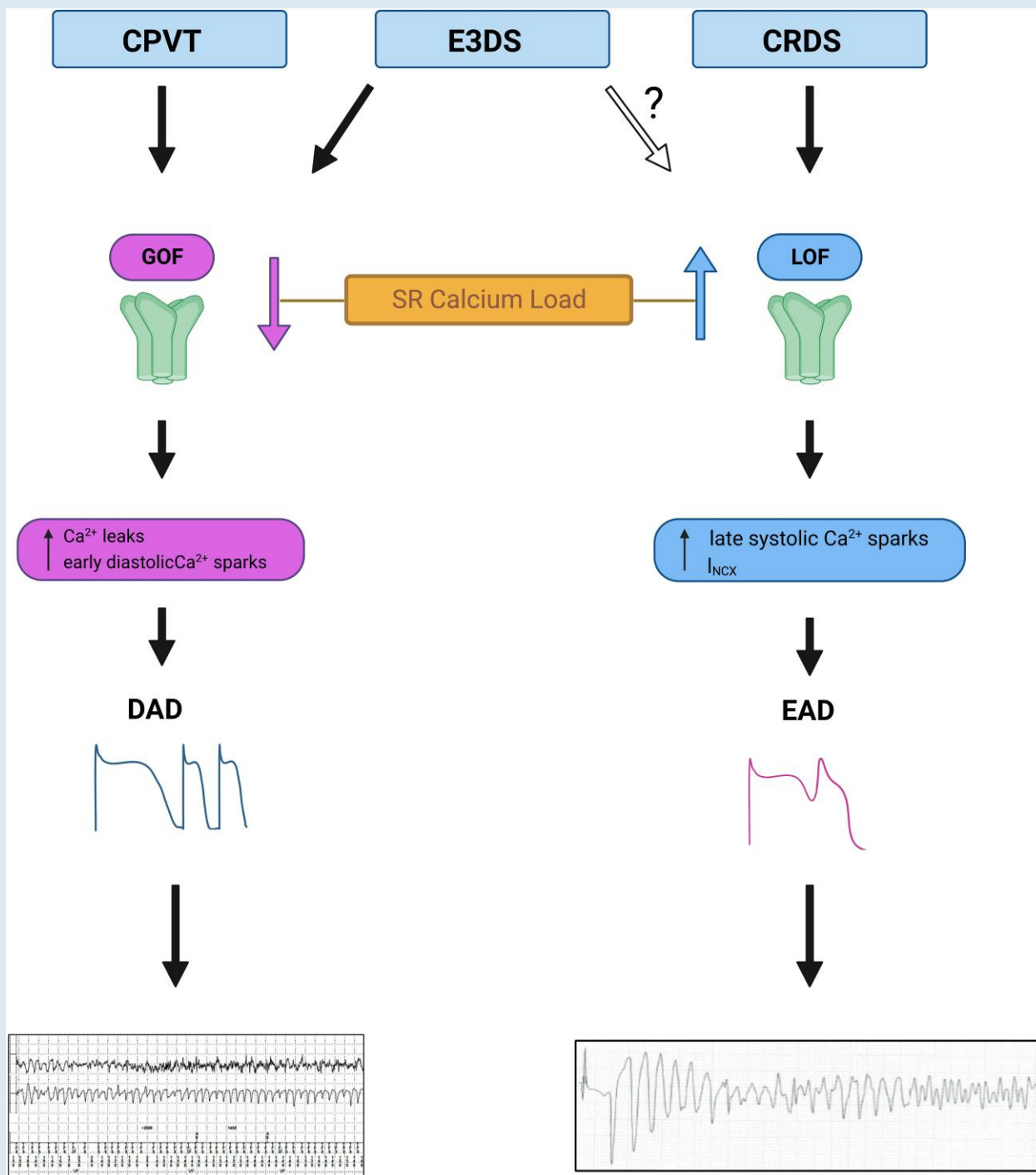
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Graphical Abstract

What's new?

- This state-of-the-art review summarizes our contemporary understanding of RYR2-related inherited arrhythmia disorders and provides a systematic and comprehensive description of the distinct cardiac ryanodinopathies that have been identified over the past decades.
- Accurate identification of the underlying type of cardiac ryanodinopathy is essential for the clinical management of affected patients and their families.

Introduction

The sarcoplasmatic reticulum (SR) cardiac ryanodine receptor/calcium release channel *RyR2* is an essential regulator of cardiac excitation–contraction coupling and intracellular calcium homeostasis.[1] The *RYR2* gene was first linked to cardiac disorders when studies by Swan *et al.* and Priori *et al.* identified *RYR2* mutations as a genetic cause of catecholaminergic polymorphic ventricular tachycardia (CPVT) more than 20 years ago.[2,3] Since that time, other distinct inherited arrhythmia syndromes have been related to abnormal *RYR2* function (*Figure 1*). The increasing number of these cardiac 'ryanodinopathies' reflects the complexity of *RYR2*-related cardiogenetic disorders and represents an ongoing challenge for clinicians. In addition to CPVT, there are at least two other distinct *RYR2*-ryanodinopathies: exon 3 deletion syndrome (E3DS) and calcium release deficiency syndrome (CRDS; *Figure 1*). The pathophysiology of the different cardiac ryanodinopathies is characterized by complex mechanisms resulting in spontaneous diastolic SR calcium release or SR calcium release deficiency.

The aim of this review is to provide an overview about the clinical aspects and molecular insights of the known cardiac ryanodinopathies.

Molecular and genetic aspects of RYR2

The cardiac ryanodine receptor (*RyR2*) is a calcium-release channel encoded by the *RYR2* gene which is located on chromosome 1 (1q42.1-q43) and comprises 105 exons.[1,4] *RYR2* translates into a large protein of 565 kDa that forms a large homotetrameric channel (~2.2 MDa) that is located within the membrane of the SR (*Figure 2*).[1,4] The N-terminal part of the protein accounts for ~90% of the entire protein mass, while the actual transmembrane pore region accounts for only ~10%.[1,5] The complex regulation of the *RYR2* channel activity is mediated by Ca^{2+} itself and numerous other modulators including complex protein interactions at both the cytosolic and intraluminal side (*Figure 2B*).[5] An in-depth discussion of the complex function and regulation of the *RyR2* channel would be beyond the scope of this article, and these aspects have been reviewed elsewhere.[6–8]

The vast majority of pathogenic *RYR2* mutations are missense variants (86–92%) (Human Gene Mutation Database version 2022.1, ClinVar April 2021).[9–11] Bioinformatic data and *in silico* tools suggest that *RyR2* poorly tolerates genetic variants that induce loss-of-function (LOF) properties.[12] However, limitations of these predictive data as well as the significant rate of genetic background noise in the healthy population are challenges that may lead to incorrect interpretation of rare *RYR2* variants.[13,14] To compensate for this problem, approaching CPVT diagnosis with a probabilistic mindset can help to overcome the estimated 3% incidence of benign heterozygous rare *RYR2* variants in the general population.[14–16] In the case of CPVT, it has been shown that using a Bayesian approach that considers the pretest probability of disease may be useful in reclassifying variants of unknown significance.[17] Keeping in mind the inherent limitations of statistical genetic methods (including Bayesian), it is important to highlight that diagnostic certainty can eventually only been achieved using a comprehensive multifactorial approach integrating statistical genetics into phenotype information (including familial segregation) and ideally functional data from cellular or animal models.

Catecholaminergic polymorphic ventricular tachycardia

Clinical aspects

Cardiac ryanodinopathies are rare disorders, with CPVT being the most common and most extensively studied phenotype. The estimated prevalence of classical CPVT is probably less than 1:10 000.[18] Catecholaminergic polymorphic ventricular tachycardia is a pure channelopathy that is not associated with structural heart disease. Ventricular arrhythmia is characterized by multifocal and polymorphic ventricular ectopy and ventricular tachycardia/ventricular fibrillation that is frequently triggered by adrenergic stimulation. Typical clinical manifestations of CPVT include syncope or sudden cardiac arrest during exercise or with emotional triggers.[18–20]

In symptomatic patients, CPVT usually manifests during childhood with a median age of disease onset at 10–11 years; however, late onset during the 3rd or 4th decade has also been reported.[21–24] Data from the international paediatric CPVT registry suggest that three quarters of symptomatic cases present with syncope and a quarter with sudden cardiac arrest.[21,23,25] A family history of unexplained sudden cardiac death in individuals younger than 40 years is present in up to 30% of probands.[20,26] However, the high symptom burden reported in many studies is often explained by proband-enriched cohorts and that the clinical spectrum of CPVT shows variable (typically mutation-dependent) phenotype expression and penetrance including a substantial proportion of more benign forms.[27] For example, large CPVT kindreds, most notably related to the R420W variant in the Netherlands and the G357S variant in the Canary Islands, demonstrate that fairly benign familial forms of CPVT exist when extensive screening is undertaken.[28,29]

Unlike other hereditary channelopathies, the resting ECG in CPVT is normal. Prominent U waves and relative, asymptomatic sinus bradycardia on resting ECGs have been reported by some authors.[19,24,30,31] Subclinical chronotropic insufficiency unmasked by exercise treadmill testing has been described in small series of paediatric patients with CPVT.[32] At least in paediatric cohorts, subclinical sinus node dysfunction off beta blocker therapy has been associated with increased ventricular arrhythmia score on treadmill testing and seems to be a risk predictor for arrhythmic events during follow-up.[32]

Exercise treadmill testing is the gold standard to unmask CPVT-related ventricular ectopy. Typical findings include a progressive burden of ventricular ectopy, with often a clear window of electrical vulnerability occurring early in exercise and before maximal exercise.[33] The typical exercise threshold to unmask ventricular ectopy or tachycardia is a heart rate window from 110–150 b.p.m.[24,33,34] With ongoing exercise, the ectopy burden may eventually decrease in a subset of CPVT patients.[35] Ventricular ectopy typically starts with isolated, late-coupled premature ventricular contractions (PVCs) with increasing complexity (multifocal and/or bidirectional PVCs, and ventricular couplets/triplets) that may result in non-sustained or sustained polymorphic or bidirectional ventricular tachycardia (*Figure 3*). Although bidirectional ventricular tachycardia has been recognized as a signature arrhythmia of CPVT (*Figure 3*), its true prevalence remains unknown; it appears much less common than previously reported.[20,23] QT dynamics at rest and during exercise are strictly normal in CPVT.[23]

In addition to ventricular arrhythmias, individuals with CPVT may also develop various types of supraventricular arrhythmias including atrial tachycardia, atrial flutter, or atrial fibrillation all reflecting the
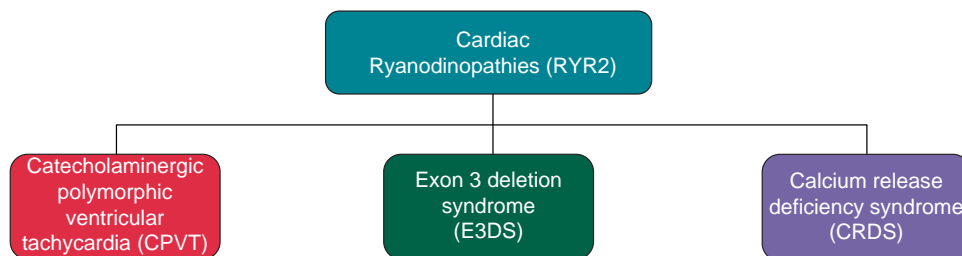
Figure 1 Cardiac ryanodinopathies. CPVT, catecholaminergic polymorphic ventricular tachycardia; CRDS, calcium release deficiency syndrome; E3DS, RYR2 exon-3 deletion syndrome.
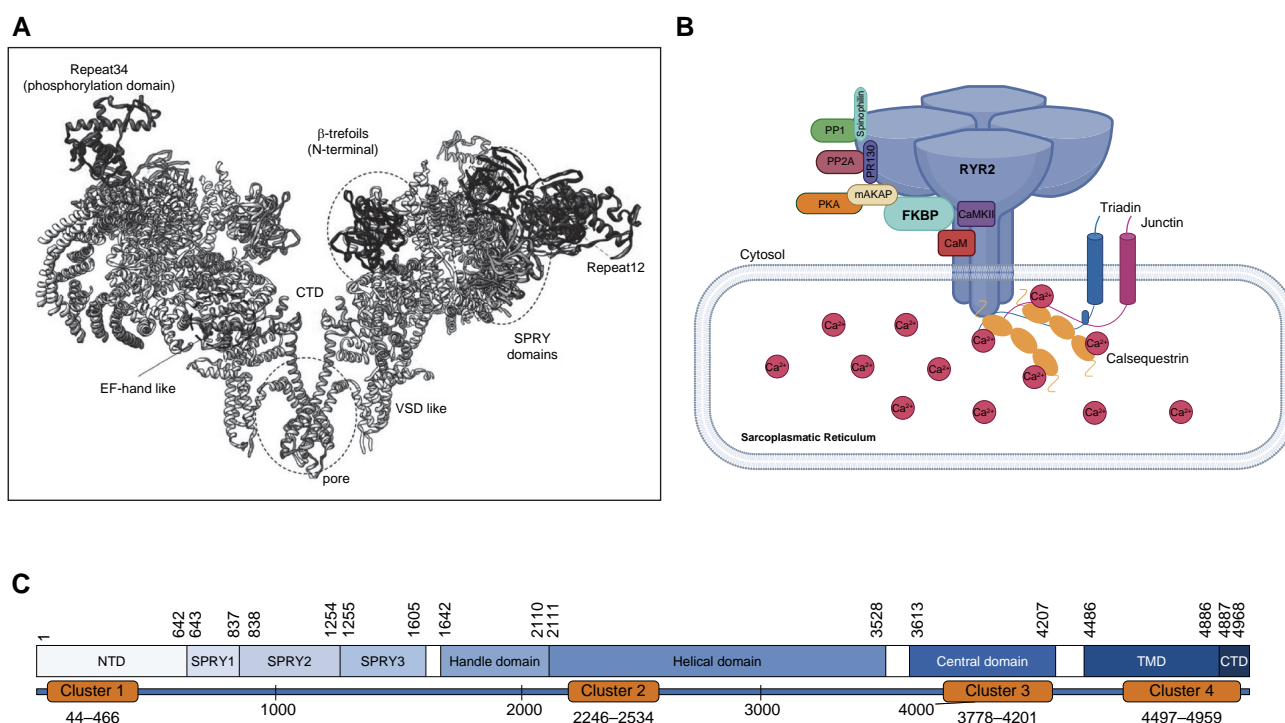
Figure 2 *RyR2* molecule. (*A*) Three-dimensional structure of *RyR2* (Protein Data Bank identification code 6JI0). (*B*) Regulation of the *RyR2* complex. CaM, calmodulin; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; FKBP, FK506-binding protein; mAKAP, muscle-specific A kinase–anchoring protein; PKA, protein kinase A; PM, plasma membrane; PP1, protein phosphatase 1; PP2A, protein phosphatase 2A; PR130, regulatory subunit of PP2A. (*C*) Schematic organization of the linear sequence of *RyR2* with major structural domains of *RyR2* (blue boxes). Orange boxes indicate four disease-associated variant clusters (variant hotspots). Adapted with permission from Zhong *et al.*[85]

underlying abnormal calcium handling.[7,19,36] In the context of CPVT, this may even result in atypical clinical scenarios such as the manifestation of atrial fibrillation during early infancy.[37]

Diagnosis of CPVT relies on appropriate phenotype assessment in conjunction with comprehensive genetic testing. Additional tools like a recently published CPVT scoring algorithm (see Supplementary material online, *Figure S1*) may facilitate the accuracy of CPVT diagnosis, in particular in suspected CPVT patients with *RYR2* variants of unknown significance.[17] Referral to a specialized cardiogenetic clinic is recommenced for all individuals with suspected or confirmed CPVT to ensure adequate diagnosis, including interpretation of genetic test results,

optimizing medical treatment, stratification for the risk of sudden cardiac death, and appropriate family screening.[38]

The cornerstone of CPVT management is appropriate beta blocker treatment. Nadolol, an unselective beta blocker, is the most effective agent to prevent breakthrough arrhythmia in CPVT and should be titrated to target daily doses of at least 1 mg/kg, but a goal of ≥1.5 mg/kg is ideal if tolerated and/or signs of CPVT are still present on exercise testing.[33,39] Nadolol access can be challenging in some countries, and propranolol is a recommended alternative.

In the case of beta blocker intolerance or failure despite adequate dosing, flecainide should be added (target dose of 2.0–3.0 mg/kg per
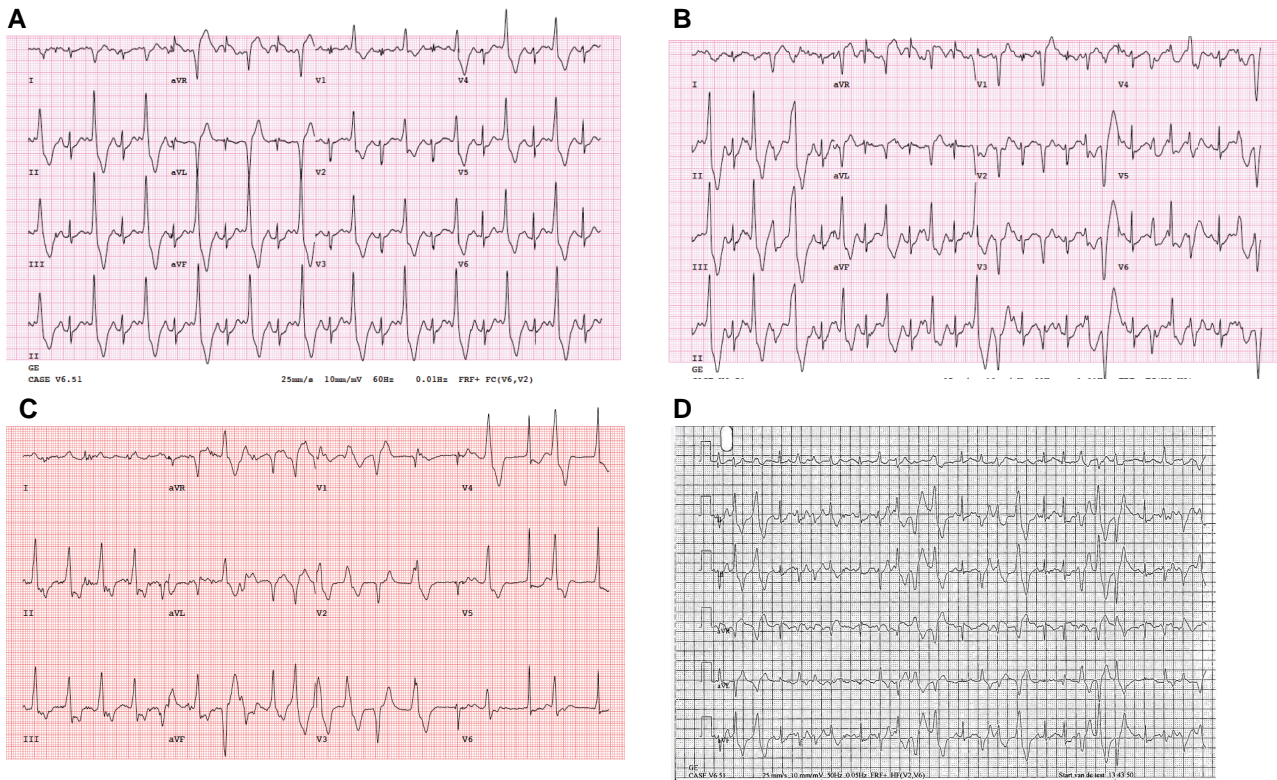
Figure 3 Catecholaminergic polymorphic ventricular tachycardia phenotype. (*A*) Exercise treatment test of a 32-year-old female patient with *RYR2*-related CPVT. Inducible ventricular ectopy with monomorphic bigeminal PVCs at Stage 2 (4:22 min) and a heart rate of 134 b.p.m. (*B*) Same patient as (*A*): at Stage 3 (6:11 min), intermittent manifestation of bidirectional PVCs. (*C*) 67-year-old male with *RYR2*-related CPVT. Run of non-sustained polymorphic ventricular tachycardia at peak Stage 4 of an exercise treadmill test (11:00 min). (*D*) Shown are intermittent runs of non-sustained bidirectional ventricular tachycardia.

day).[28,30,40,41] Some patients will experience cardiac breakthrough events despite optimized antiarrhythmic medication. Invasive treatment options include left cardiac sympathetic denervation (LCSD) that should be considered in individuals with drug refractory CPVT, intolerance or non-adherence to drug therapy, and some very high risk individuals in addition to standard medication.[42–44] When performed by experienced centres, LCSD is highly effective, reducing the risk of major cardiac events by 70–90% and the risk of appropriate implantable cardioverter defibrillator (ICD) shocks by 93%.[44–47]

Exercise stress testing is integral to optimizing these therapies. Early data showed that suppression of ventricular ectopy during exercise testing was associated with a lower risk of events and that over time, serial exercise testing results are generally reproducible.[39,48] Emerging data also suggest that an exercise stress test protocol involving an initial sprint may be more sensitive and could be useful to confirm adequate arrhythmia suppression after therapies appear to be optimized.[49]

The role and benefits of ICD insertion in CPVT remain controversial. Current guidelines recommend ICD insertion for secondary prevention after resuscitated sudden cardiac arrest or for primary prevention in individuals with arrhythmic syncope while on appropriate antiarrhythmic medication.[43,44] Special considerations may apply to paediatric CPVT patients given their particular vulnerability to device-related complications in the short and long term. The 2021 PACES Expert Consensus Statement on the Indications and Management of Cardiovascular Implantable Electronic Devices in Pediatric Patients suggest that 'In selected patients with aborted SCA as the initial presentation of CPVT,

pharmacologic therapy and/or cardiac sympathetic denervation without ICD may be considered as a possible alternative.'[50]

In the absence of prospective, large-scale studies, the question of a survival benefit in recipients of a secondary prevention ICD remains unanswered. Several studies reported no survival benefit and a high rate of device-related complications including inappropriate shocks in 20–25% and hardware-related complications in 29–32% over a median follow-up of 4–5 years.[51–53] This is in contrast to the findings of another group reporting lower rates of device-related complications and a potential survival benefit.[54] Appropriate ICD therapies typically occur in patients without appropriate antiarrhythmic medication.[51] On the other hand, 1–4% of CPVT patients experience sudden cardiac death despite an ICD.[51,52] This is in part due to shocks that are a powerful adrenergic trigger for recurrent polymorphic VT and subsequent degeneration to ventricular fibrillation, potentially leading to exhaustion of available ICD shock delivery (typically after six shocks). Overall, contemporary data suggest the importance of a careful and comprehensive patient evaluation in an expert setting with shared decision-making to avoid unnecessary, potentially harmful ICD implantation.[55] If ICD implantation is inevitable, it is important to ensure adequate device programming including long detection delays and high cut-off rates for shock delivery to minimize the risk of inappropriate shocks and enabling spontaneous termination. In the absence of prospective ICD data in CPVT patients, the suggestions of device programming are extrapolated and adapted from the general concepts of adequate contemporary ICD programming.[56,57]

Table 1 Arrhythmogenesis and cellular electrophysiological findings of cardiac ryanodinopathies

	Caffeine-induced Ca²⁺ release	SOICR	Isoproterenol stimulation	DAD	EAD
CPVT	↑↑	↑↑	↑↑ DAD + ventricular arrhythmia	+	−
Exon 3 deletion syndrome	ND	Impaired termination	↑↑ DAD + ventricular arrhythmia?	+	−
CRDS	↓↓	↓↓	−	−	+

CPVT, catecholaminergic polymorphic ventricular tachycardia; CRDS, calcium release deficiency syndrome; DAD, delayed afterdepolarization; EAD, early afterdepolarization; ND, not determined; SOICR, store overload-induced Ca²⁺ release.

Table 2 Clinical features and ECG characteristics of cardiac ryanodinopathies

	QTc[a]	Exercise-induced ventricular ectopy	Syncope/presyncope prior to sudden cardiac arrest	Bidirectional VT
CPVT	Normal	Common	Common	Yes
Exon 3 deletion syndrome	Normal	Common	Possible	Yes
CRDS	Normal	Infrequent	Possible	No

CPVT, catecholaminergic polymorphic ventricular tachycardia; CRDS, calcium release deficiency syndrome; QTc, corrected QT interval.
[a]At rest and during/post exercise.

Genetic and electrophysiological aspects

Pathogenic *RYR2* variants account for at least half but probably over 80% of all CPVT cases, and transmission is typically autosomal-dominant.[21,58] In addition, there are rare cases of *RYR2*-related CPVT with autosomal-recessive transmission.[59] Other genetic substrates of CPVT have been extensively reviewed elsewhere and will not be discussed in this article.[7,58] The majority of CPVT-associated *RYR2* mutations are missense variants (96%) resulting in a gain-of-function (GOF) of the *RyR2* channel.[7,58] The distribution of CPVT *RYR2* variants shows a clustering within four distinct mutational hotspot regions including exons 3–15 (amino acids 44–466), 44–50 amino acids (2246–2534), 83–90 (amino acids 3778–4201), and exons 93–105 (amino acids 4497–4959) (*Figure 2C*).[15,25,60] Three of these cluster regions are located within the N-terminal portion of the *RYR2* protein. Only 10% of CPVT-related variants are located outside these mutational hotspot regions.[15] A substantial proportion of monogenetic *RYR2*-related CPVT cases is related to *de novo* variants.[27,61,62] Interestingly, *RYR2 de novo* variants are more likely to be located within the C-terminus domain compared to familial *RYR2* variants (more likely within the N-terminal domain).[62] Limited data suggest that disease manifestation in probands harbouring *de novo* variants is earlier and phenotype traits seem to be more severe compared to probands with familial forms of CPVT and *RYR2* variants at other locations.[62] Interestingly, an association between C-terminal *RYR2* variants and increased risk for ventricular arrhythmia has also been observed in familial forms of CPVT.[25,27,54] Disease penetrance is incomplete and may be mutation-dependent. Previous studies suggested a penetrance of 50–65%.[27,63]

The arrhythmogenesis of CPVT is complex and implies different, mutation-dependent mechanisms affecting the Ca²⁺ activation, protein folding, or binding sites of regulatory proteins of *RyR2*.[7,18,64] At the cellular level, *RYR2* GOF variants result in abnormal calcium handling with spontaneous diastolic calcium release which in turn triggers delayed afterdepolarizations that initiate ventricular arrhythmia.[7,65,66] Corresponding cellular electrophysiological findings of CPVT are depicted in *Tables 1* and *2* and in the *Central Illustration*. One important

mechanism is mediated by a reduced threshold for the occurrence of store overload-induced Ca²⁺ release (SOICR) which is favoured by an increased sensitivity of the *RyR2* channel to the activation by luminal and/or cytosolic Ca²⁺ (*Central Illustration*).[67,68] Typical conditions leading to increased sarcoplasmic reticulum calcium load include stimulation with catecholamines, for example, isoproterenol.[65,66] Another typical cellular electrophysiological finding of abnormal calcium handling is the enhanced response to caffeine-induced calcium release from the sarcoplasmic reticulum.[66,68,69] Another prerequisite for the arrhythmogenesis in CPVT is the existence of an arrhythmic heart rate window as described above that determines the balance of SR calcium loading and spontaneous diastolic calcium release.[35] Experiments in mice and humans showed that increasing the sinus rate through vagolytic pretreatment with atropine or atrial overdrive pacing significantly reduces the occurrence of exercise-induced ventricular arrhythmia.[35,70] A likely explanation for this observation is that shortening the diastole prevents spontaneous SR calcium release.[7,70]

RyR2 exon 3 deletion syndrome
Clinical aspects

First described in 2007,[71] the *RyR2* E3DS has now been recognized as a distinct entity among the different cardiac ryanodinopathies.[72–77] With less than 50 cases published to date, E3DS represents a very rare *RYR2*-ryanodinopathy. Assuming that deletion of exon 3 accounts for ~1% of pathogenic *RYR2* variants, the prevalence of E3DS might be estimated at 1:100 000.

In contrast to the exclusive tachyarrhythmias of other cardiac ryanodinopathies, E3DS is characterized by a complex pleiotropic tachycardia–bradycardia phenotype (*Table 3*). The mixed phenotype shows marked interindividual variability within affected families. A typical example of the marked phenotype variability is displayed in *Figures 4* and *5*, showing examples from a large French–Canadian family affected by E3DS.

Given its rarity and the paucity of published data, the frequency of the various electrophysiological and structural features of E3DS can
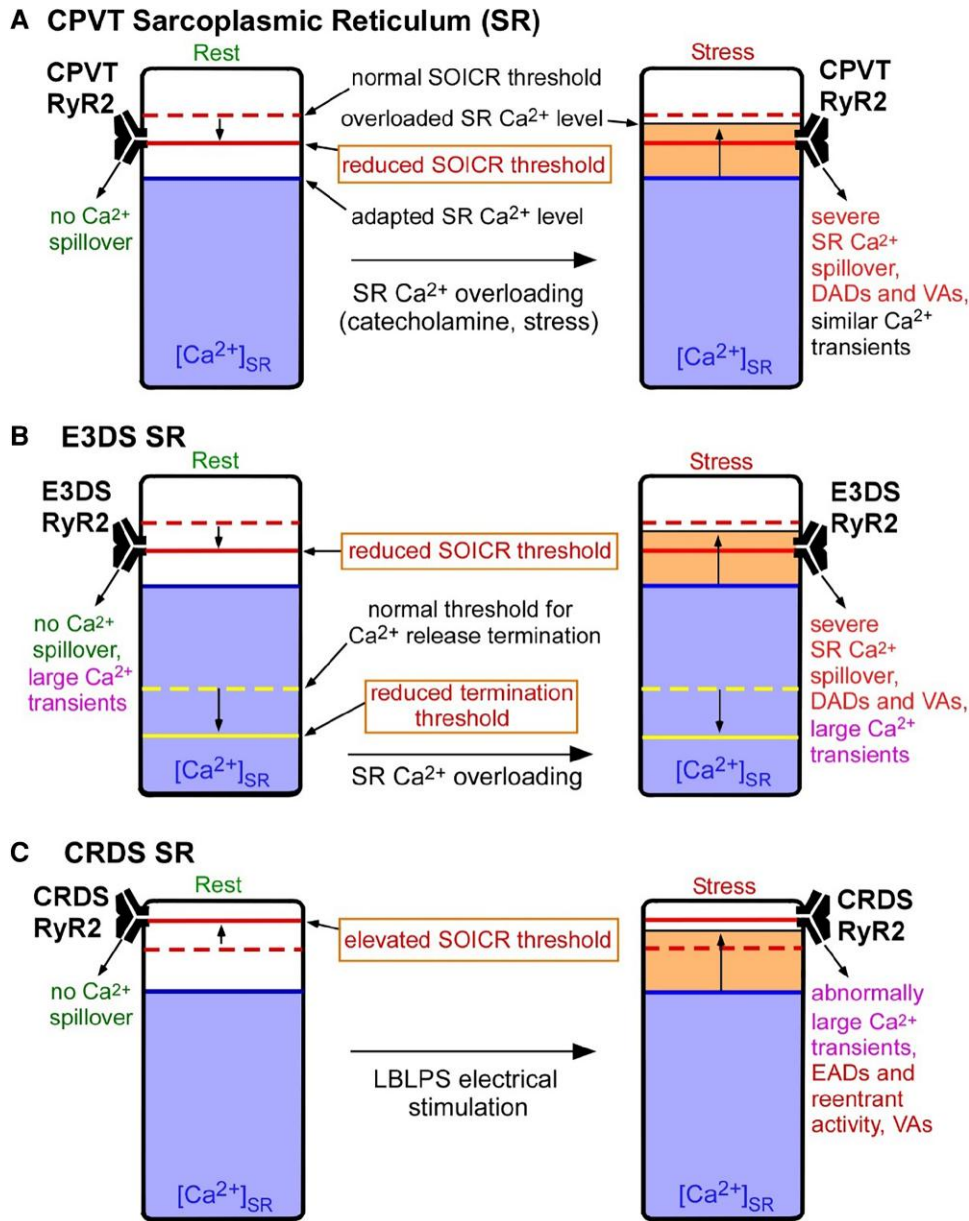
Central Illustration Proposed mechanisms for *RyR2*-associated catecholaminergic polymorphic ventricular tachycardia (CPVT), exon 3 deletion syndrome (E3DS), and calcium release deficiency syndrome (CRDS). The different thresholds for store overload-induced Ca^{2+} release (SOICR) and Ca^{2+} release termination and the free sarcoplasmic reticulum (SR) luminal Ca^{2+} levels in CPVT, E3DS, or CRDS associated with *RyR2* mutations are illustrated in the resting state (Rest, left panels) and in the stress states (Stress, right panels). The normal thresholds for SOICR and Ca^{2+} release termination are depicted as red and yellow dashed bars, respectively. The reduced or elevated SOICR thresholds as a consequence of CPVT, E3DS, or CRDS RyR2 mutations are depicted as solid red bars. The reduced threshold for Ca^{2+} release termination as a consequence of E3DS *RyR2* mutations are depicted as solid yellow bars. The SR free luminal Ca^{2+} level is represented as a blue area. The yellow areas above the blue areas in the right panels represent an elevation, even if only transient, in the free SR luminal Ca^{2+} levels, which, we propose, will occur when sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) activity is enhanced by catecholamines or during the long-burst, long-pause, short-coupled (LBLPS) programed electrical stimulation. When the SR-free luminal Ca^{2+} level surpasses, even transiently, the reduced SOICR threshold in the case of CPVT and E3DS, SOICR occurs, leading to a spillover of SR Ca^{2+} that can trigger spontaneous Ca^{2+} release, delayed afterdepolarizations (DADs) and ventricular arrhythmias (VAs). The reduced termination threshold in the case of E3DS will increase the fractional Ca^{2+} release, resulting in large Ca^{2+} transients at rest (left panels) and stress (right panels) that may promote cardiomyopathies in addition to cardiac arrhythmia. In the case of CRDS, the elevated SOICR threshold prevents spontaneous SR Ca^{2+} leak, leading to markedly elevated SR Ca^{2+} load upon LBLPS electrical stimulation and subsequently large Ca^{2+} transients that promote early afterdepolarizations (EADs), reentrant activity, and ventricular arrhythmias.

Table 3 Phenotype findings in E3DS

Clinical findings in RYR2 exon 3 deletion syndrome[a]	
Sinus node dysfunction	58%
Atrial standstill	7%
AV node conduction disorders[b]	22%
Atrial fibrillation/atrial flutter	18%
Atrial tachycardia or other SVT	36%
CPVT-like ventricular arrhythmias	56%
Sudden cardiac death	11%
Dilated cardiomyopathy ± left ventricular non-compaction	18%
Mitral valve prolapse	4%

AV, atrioventricular; CPVT, catecholaminergic polymorphic ventricular tachycardia; SVT, supraventricular tachycardia.

[a]All estimates are based on a pooled analysis of published reports and the first author's own cohort of E3DS patients. The definition of the different rhythm disorders and cardiomyopathies is based on current guidelines. Only rhythm disorders with appropriate clinical testing and description in the corresponding articles were retained for data collection and analysis. Given the variable extent of clinical testing and limited follow-up data in the reviewed literature, the pooled findings may only provide crude estimates of the frequency of E3DS-related rhythm disorders.

[b]Including second-degree, third-degree, or high degree AV block.

only be estimated. *Table 3* depicts the spectrum of relevant clinical features associated with E3DS. Sinus node dysfunction manifesting with symptomatic sinus bradycardia, chronotropic insufficiency, sinus arrest, or atrial stillstand represents the leading bradyarrhythmia in E3DS (58%) (*Table 3*). Atrioventricular (AV) node conduction disorders have been described in up to 22% of gene carriers. Atrial fibrillation/flutter and other supraventricular tachycardias are common and will develop in more than half of affected patients over time. Ventricular arrhythmia is characterized by a CPVT including exercise-triggered ventricular ectopy, bidirectional ventricular tachycardia, as well as polymorphic VT/ventricular fibrillation.[71–75,77,78] A family history of sudden cardiac death is present in at least 10% of affected individuals.

Isolated left ventricular non-compaction (LVNC) without ventricular enlargement or left ventricular systolic dysfunction has been reported in up to 31% of patients with E3DS.[75,78] True dilated cardiomyopathy with or without LVNC seems to be less common and is observed in up to 18% of cases (*Table 3*). A novel but potentially underreported finding is the co-association of mitral valve prolapse that was observed our French–Canadian cohort (*Figure 5*).

The mixed tachycardia–bradycardia phenotype of E3DS represents a major challenge for pharmacologic management. Patients with E3DS are extremely sensitive and often intolerant to various antiarrhythmic or heart rate slowing medication (personal, unpublished observations), which is typically required to treat the CPVT phenotype and the common supraventricular arrhythmias. Even very small doses of cardioselective beta blockers or calcium channel blockers may result in excessive symptomatic bradycardia (personal unpublished observation).[76,77] Consequences of the marked pharmacological intolerance and the frequent severe sinus node dysfunction include early insertion of cardiac implantable electronic devices. Appropriate device selection may be challenging and requires a thorough and comprehensive evaluation as most E3DS patients will become recipients at young age.

Genetic and electrophysiological aspects

The deletion of exon 3 (c.161 to c.272) and the subsequent genetic rearrangement result in an in-frame deletion of 35 amino acids of the *RyR2* N-terminal domain A (p.Asn 57_Gly91del; NM_001035)

(*Figure 4A*).[71,79] The estimated size of genomic deletion is 241 bp affecting the entire exon 3 and the flanking introns 2 and 3.[71,78] The molecular mechanisms leading to exon 3 deletion remain incompletely understood. Bhuiyan and coworkers proposed polymerase slippage as a result of *Alu-I* transposon repeats.[71]

Structural modelling suggests that the altered *RyR2* protein facilitates pore opening and in turn diastolic spontaneous calcium release.[79] At the cellular level, E3DS increases the propensity for SOICR by reducing the threshold for SOICR activation. Exon 3 deletion syndrome also increases the amplitude of SOICR by impairing calcium release termination or disinhibition of the *RyR2* channel (*Central Illustration*).[80]

Exon 3 deletion syndrome shows full penetrance but variable disease expression. So far, no homozygous gene carriers and no asymptomatic carriers of E3DS have been reported. The marked phenotype variability even within a single family (*Figure 5*) may be related to yet unknown genetic modifiers and/or epigenetic factors. Transmission of E3DS is typically autosomal dominant, but *de novo* events have also been described.[76] The age of onset is highly variable and can be as young as 7 years. Virtually all gene carriers will present with some phenotype traits by 45 years of age (personal unpublished observations). The totality of the data strongly supports classifying E3DS as a unique overlap of inherited arrhythmia and cardiomyopathy that is distinct from CPVT.

RyR2 calcium release deficiency syndrome
Clinical aspects

The *RyR2* CRDS represents a novel, emerging cardiac ryanodinopathy and has only recently been characterized.[81–84] Given its infancy as an established syndrome and the challenges surrounding diagnostic testing that will be discussed, the prevalence of CRDS is unknown. Average age of presentation appears to be in early adulthood, but index events during childhood and preadolescence are not uncommon.[82]
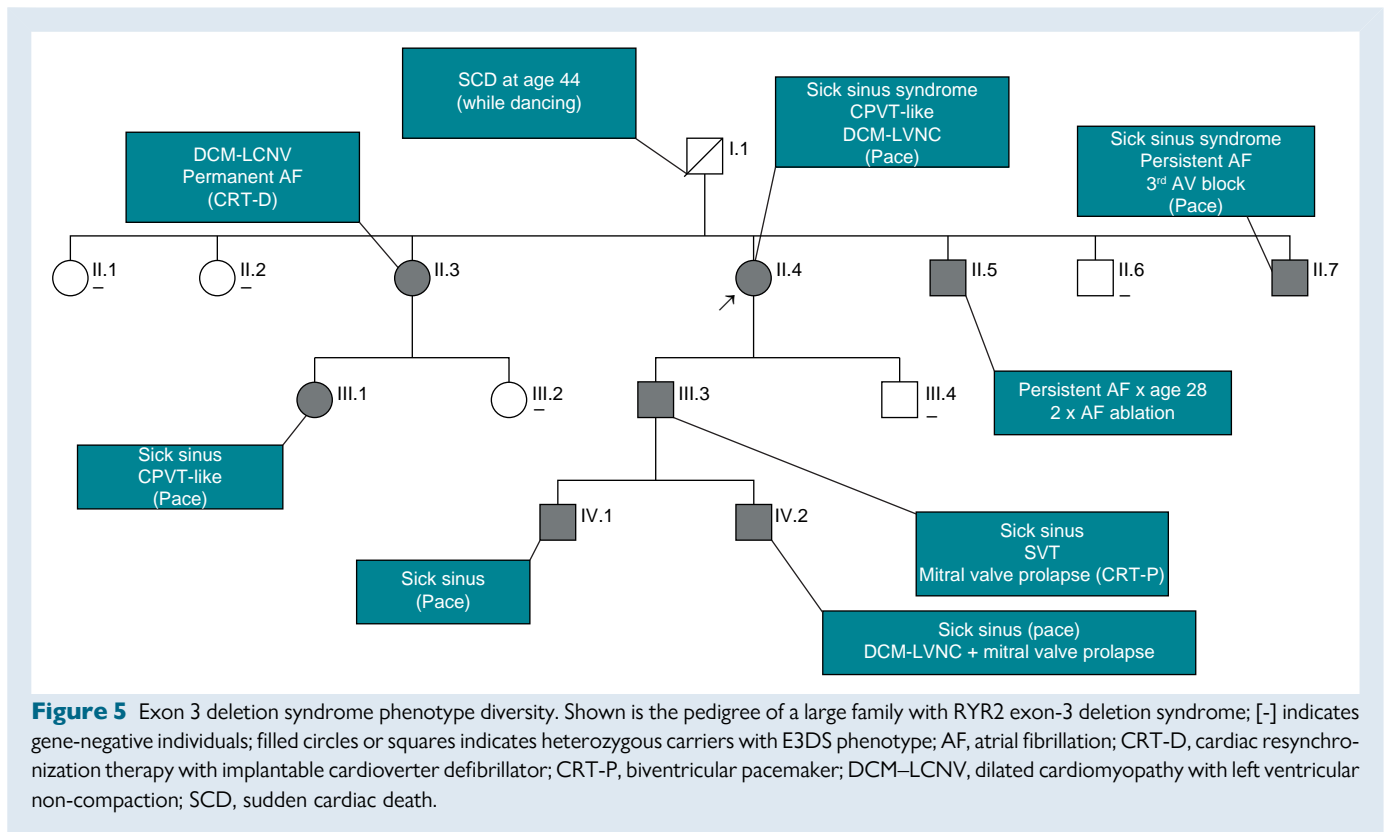
Despite some overlapping clinical features, there is growing evidence that CRDS is a phenotypically and mechanistically distinct *RYR2*-related channelopathy that differs from classic CPVT. Individuals with CRDS are susceptible to malignant ventricular arrhythmia (polymorphic ventricular tachycardia and ventricular fibrillation) resulting in syncope or sudden cardiac death.[81–85]

Unlike classic CPVT, only 50% of arrhythmic events in CRDS seem to occur during adrenergic stimulation.[82] Similar to CPVT, the resting ECG is normal in CRDS, although one group has suggested that some patients may also exhibit mild corrected QT interval (QTc) prolongation.[86] In contrast to CPVT, exercise treadmill testing has limited diagnostic value in CRDS and does not usually show complex polymorphic ventricular ectopy or bidirectional ventricular tachycardia.[81–84] In the largest cohort of CRDS patients described so far (19 patients including probands and relatives), exercise treadmill testing was negative in 64% and showed only isolated ventricular PVCs in 34%.[82] This lack of sensitivity is most likely related to the particular electrophysiological mechanisms that have been identified in CRDS (see below). The same mechanisms may also account for the incomplete penetrance and an overall lower rate of cardiac breakthrough events compared to classic CPVT.[81,82] Sudden cardiac arrest or sustained ventricular arrhythmia occur in at least 23% of individuals with CRDS, though there is clear ascertainment bias in this estimate.[82]

The most accurate clinical test to unmask CRDS is a specific protocol of programmed ventricular stimulation characterized by a long-burst, long-pause, and short-coupled ventricular extra stimulus (LBLPS) sequence (Figure 6A, B).[81–83] Current data on the sensitivity and specificity of a dedicated LBLPS protocol are still limited. Initial experience in a limited number of individuals showed inducible ventricular arrhythmia in up to 78% of CRDS patients.[81] The LBLPS sequence also reflects the arrhythmia onset of spontaneous events (*Figure 6C*).[82] There is also emerging evidence that the same protocol may be useful to determine the efficacy of antiarrhythmic medication.[81]
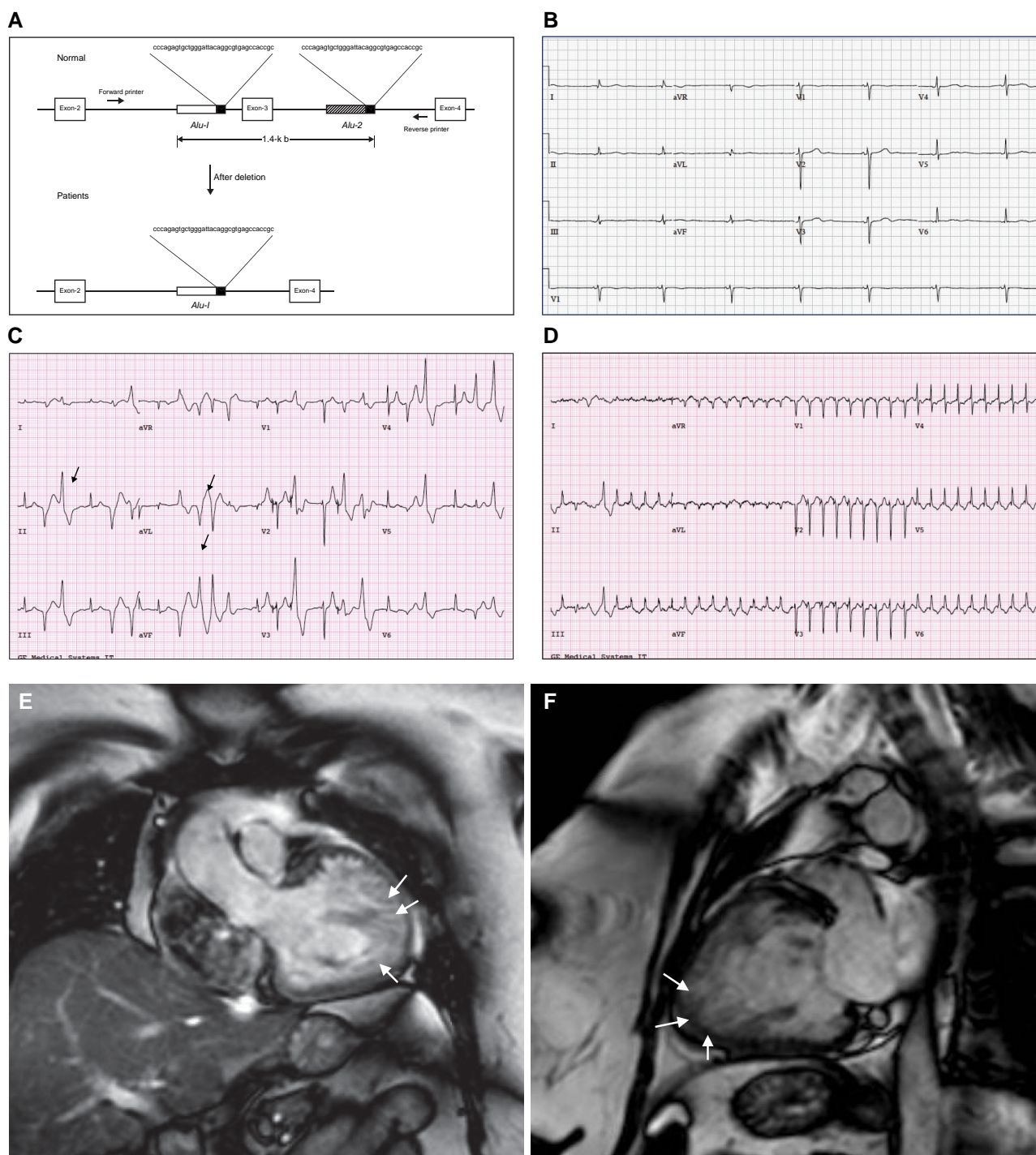
Figure 4 Exon 3 deletion syndrome clinical phenotype. (*A*) *Alu* repeat–mediated RYR2 exon 3 deletion. The diagram represents the Alu-Alu recombination. Alu sequences are located in intron 2, 190 bp upstream from exon 3 and also 536 bp downstream in intron 3. Adapted with permission from Bhuiyan *et al.*[71] (*B*) 34-year-old female patient with E3DS. Resting ECG showing marked junctional bradycardia at 42 b.p.m. The patient had symptomatic sinus node disease with chronotropic insufficiency. (*C*) 48-year-old female patient with E3DS. Exercise treadmill testing showed inducible polymorphic ventricular ectopy with intermittent bidirectional ventricular PVCs (arrows). (*D*) Same patient as (*C*). Induction of supraventricular tachycardia at peak exercise. The echocardiogram showed a normal sized left ventricular with preserved ejection fraction. However, there was evidence of marked apical and apico-lateral trabeculation meeting criteria for LVNC. (*E*) and (*F*) Cardiac magnetic resonance imaging of a 52-year-old female patient with E3DS. The patient developed dilated cardiomyopathy with LVNC and marked systolic dysfunction requiring the insertion of a cardiac resynchronization therapy defibrillator. Note the marked left ventricular apical and apico-lateral hypertrabeculations (arrows).

Figure 5 Exon 3 deletion syndrome phenotype diversity. Shown is the pedigree of a large family with RYR2 exon-3 deletion syndrome; [-] indicates gene-negative individuals; filled circles or squares indicates heterozygous carriers with E3DS phenotype; AF, atrial fibrillation; CRT-D, cardiac resynchronization therapy with implantable cardioverter defibrillator; CRT-P, biventricular pacemaker; DCM–LCNV, dilated cardiomyopathy with left ventricular non-compaction; SCD, sudden cardiac death.

Data on the optimal medical management in CRDS remain sparse. Unlike CPVT, the benefit of beta blockers is less certain with break-through events in ~20%,[81,82] and experience with this class of medication almost exclusively stems from the initial misdiagnosis of CPVT that occurs in most patients. Potential antiarrhythmics include flecainide and quinidine, which effectively suppress ventricular fibrillation in a CRDS mouse model.[83] Flecainide monotherapy also suppressed LBLPS-inducible ventricular arrhythmia in 89% of human adults with CRDS.[81] In contrast, the combination of flecainide with metoprolol seems to abolish the protective effect of flecainide in a significant proportion of patients; however, convincing follow-up data supporting these findings are yet missing.[81] Clearly, further studies with larger cohorts and longer follow-up periods will be required to identify the best antiarrhythmic treatment strategies in CRDS. In addition to the knowledge gaps with regard to medical treatment, the role of primary prevention ICDs in CRDS remains undefined at this point.'

Genetic and electrophysiological aspects

The genetic substrate of CRDS is *RYR2* mutations resulting in LOF. These LOF variants are predominantly located in the C-terminal Cluster 4 region, which is also a well-known mutational hotspot for CPVT.[15,60,81–84] The vast majority of CRDS-related mutations are *RYR2* missense variants with autosomal dominant transmission. An exception is a particularly aggressive form of autosomal recessive CRDS that has been linked to a copy number variant mutation of *RYR2*.[84] The tandem duplication including the 5′ untranslated region of the *RYR2* promoter region and exons 1 through 4 has only been identified in Amish communities, so far.[84] The resulting haploinsufficiency translates into a highly penetrant and lethal phenotype (78% of sudden cardiac arrest or death).[84,87]

With the exception of this very aggressive phenotype, current data on other CRDS-associated variants do not yet allow the determination of mutation-dependent disease severity. Unlike CPVT, CRDS has only

been linked to *RYR2*, and no other genetic substrates have been described, so far.

Functional data from cellular and animal models suggest an arrhythmogenesis distinctly different from CPVT. Loss-of-function is characterized by a significant decrease of the propensity for stress-induced ventricular arrhythmia upon stimulation with caffeine or isoproterenol compared to wild type *RyR2* channels.[81–83,85] In a similar way, SOICR is markedly inhibited resulting in a decreased propensity for delayed afterdepolarizations (*Central Illustration*).[81] Additional findings of the electrophysiological remodelling include increases of I_{Ca-L}, I_{to}, and I_{NCX} currents and a hyperpolarization shift of the voltage-dependent activation of the cardiac sodium channel $Na_v1.5$.[83,88] The result is a prolonged duration of the cardiac action potential with increased propensity to early afterdepolarizations that may trigger reentrant ventricular arrhythmia.[83,87,88]

Other cardiac ryanodinopathies and overlap features

In addition to the distinct phenotypes of the classical *RyR2* CPVT, CRDS, or E3DS, there is some evidence suggesting the existence of other forms of yet unclassified cardiac ryanodinopathies with atypical phenotypes and unusual electrophysiological properties[85,86,89–92] (see Supplementary material online, *Table S2*). Although data on these atypical cardiac ryanodinopathies are restricted to case reports with limited segregation and clinical follow-up, the published reports highlight the incredible complexity of *RYR2*-related inherited arrhythmia syndromes. Many questions still remain unanswered. Some of the cardiac ryanodinopathies that present with atypical abnormalities on exercise testing may represent subtypes of CRDS, given that not all LOF carriers exhibit identical phenotypes. Thus, the clinical spectrum of cardiac ryanodinopathies may be even broader than appreciated, with disease entities yet to be discovered. These concepts suggest that both the amount and timing of calcium release and inhibition from *RyR2* play an integral role in the maintenance of cardiac rhythm and structure and thus is process prone to marked pleiotropy.
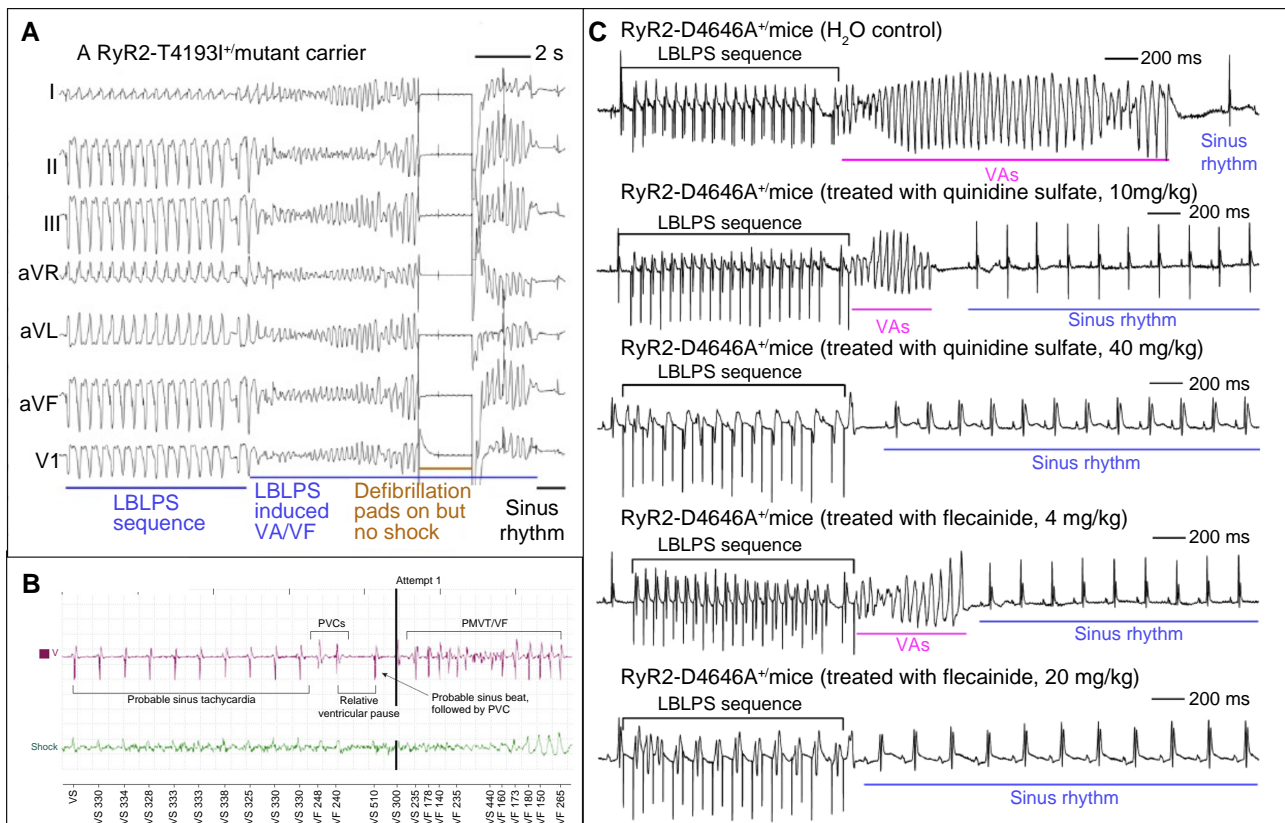
Figure 6 Ventricular arrhythmia induction in CRDS. (*A*) Ventricular fibrillation induction in CRDS using programmed ventricular stimulation. Shown are the ECG tracings of a CRDS proband carrying the *RYR2*-T4196I LOF variant. Ventricular arrhythmia is reproducibly precipitated using the LBLPS stimulation protocol (adapted with permission from Sun et al.[83]). (*B*) Spontaneous VF initiation in CRDS. Implantable cardioverter defibrillator tracings from a CRDS proband harbouring a *RYR2* LOF variant. Shown is an episode of ventricular fibrillation preceded by sinus tachycardia. Ventricular tachycardia is then initiated by a sequence of two premature ventricular complexes, a long pause, a sinus beat, and a shorter coupled PVC (adapted with permission from Roston et al.[82]). (*C*) Effects of quinidine and flecainide on VA in D4646A +/− mutant mice. The tracing in the top row displays typical VF induction using a LBLPS stimulation (mice treated with H_2O control). Tracings from the second and third row demonstrate a dose-dependent (10 mg/kg vs. 40 mg/kg per day) suppression of inducible ventricular arrhythmia after pretreatment with quinidine sulphate for 6 days. Similar effects on VA inducibility were also observed in mice after pretreatment with different doses (4 mg/kg vs. 20 mg/kg per day) of flecainide for 6 days. LBLPS, long-burst, long-pause, and short-coupled ventricular extra stimulus; PVC, premature ventricular contraction; VA, ventricular arrhythmia; VF, ventricular fibrillation (adapted with permission from Sun et al.[83]).

Future directions

Large-scale international registries exist for CPVT and have provided the basis for much of our knowledge surrounding this condition. Similar collaborative approaches are also required for other cardiac ryanodinopathies to improve our understanding of the different phenotypes and clinical evolution of disease. Larger cohorts are also fundamental to design future studies to shift our current approach towards genotype-based disease management.[93] Refined disease modelling incorporating novel technologies such as three-dimensional human-engineered heart tissue may provide us with complementary functional data for tailored therapies.[94,95] Human inducible pluripotent stem cell (hiPSC) platforms are already increasingly being used for CPVT research with similar utility anticipated for other cardiac ryanodinopathies.[96–99] Promising pathways for potential future curative gene therapy may include allele-specific gene silencing and targeted *in vivo* gene editing using viral vectors.[100,101]

Complementary functional genomic studies in conjunction with data from cellular models and clinical registries may help us to identify additional genetic and non-genetic cofactors that affect disease severity and prognosis.

In addition to gene-therapeutic strategies, a number of novel *RyR2*-specific pharmacological therapies are currently under investigation, although very few of them have entered the stage of clinical testing so far. One of them is the oral *RyR2* modulator S48168 (ARM210) that will be assessed in CPVT patients in an upcoming Phase II trial starting in 2023 (NCT05122975). Other molecules with pharmacological properties for potential *RyR2*-directed precision medicine like EL20, K201, or *ent*-(+)-verticilide are still at the preclinical stage.[102–105]

Supplementary material

Supplementary material is available at *Europace* online.

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