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Review article

# Ryanodine receptors and ventricular arrhythmias: Emerging trends in mutations, mechanisms and therapies

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**Abstract**

It has been six years since the first reported link between mutations in the cardiac ryanodine receptor $Ca^{2+}$ release channel (RyR2) and catecholaminergic polymorphic ventricular tachycardia (CPVT), a malignant stress-induced arrhythmia. In this time, rapid advances have been made in identifying new mutations, and in understanding how these mutations disrupt normal channel function to cause VT that frequently degenerates into ventricular fibrillation (VF) and sudden death. Functional characterisation of these RyR2 $Ca^{2+}$ channelopathies suggests that mutations alter the ability of RyR2 to sense its intracellular environment, and that channel modulation via covalent modification, $Ca^{2+}$- and $Mg^{2+}$-dependent regulation and structural feedback mechanisms are catastrophically disturbed. This review reconciles the current status of RyR2 mutation-linked etiopathology, the significance of mutational clustering within the RyR2 polypeptide and the mechanisms underlying channel dysfunction. We will also review new data that explores the link between abnormal $Ca^{2+}$ release and the resultant cardiac electrical instability in VT and VF, and how these recent developments impact on novel anti-arrhythmic therapies. Finally, we evaluate the concept that mechanistic differences between CPVT and other arrhythmogenic disorders may preclude a common therapeutic strategy to normalise RyR2 function in cardiac disease.


*Keywords:* Ryanodine receptor; Mutation; Arrhythmia; Sudden cardiac death; CPVT; $Ca^{2+}$ release; Interdomain interactions; Mechanism

**Contents**



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## 1. Introduction

"When the heart is diseased, its work is imperfectly performed: the vessels proceeding from the heart become inactive, so that you cannot feel them … if the heart trembles, has little power and sinks, the disease is advanced and death is near."

This description, found in the Ebers Papyrus of Ancient Egypt pre-dating 1500 BC, is thought to be the earliest recorded account of ventricular fibrillation (VF). We now understand many of the molecular mechanisms that lead to the disruption of normal heart rhythm (arrhythmia) and its deterioration into a catastrophic breakdown of electrical synchrony (VF), the main cause of sudden death (SD). Despite our knowledge of many of these underlying defects, SD remains a major cause of mortality accounting for more than 750,000 deaths per year in Europe and the US (~0.1% of total recorded deaths) [1].

Arrhythmia results from perturbation of the exquisitely controlled fluxes of $Na^+$, $K^+$ and $Ca^{2+}$ ions both within cardiomyocytes and between the myocardium and the external milieu [2]. These ionic fluxes are highly interdependent, and thus localised disruption may exacerbate global ion flux imbalance resulting in the complete ablation of synchronous cardiac electrical activity. Over the last two decades, defective mechanisms in arrhythmogenic ion fluxes have been elucidated by functional characterisation of $Na^+$ and $K^+$ channels containing genetic mutations ('channelopathies') [3–5]. Currently, the genetic basis of $Ca^{2+}$ handling dysfunction in arrhythmia is the focus of widespread attention, and is the subject of this review.

Intracellular and trans-plasmalemmal $Ca^{2+}$ fluxes coordinate multiple facets of cardiac function [2,6,7], and precisely controlled $Ca^{2+}$ cycling is a prerequisite for normal cardiac rhythm and contractility. Genetic mutations underlying malignant arrhythmias have recently been identified in cardiac $Ca^{2+}$ channels including the L-type $Ca^{2+}$ channel (LTCC, also termed the dihydropyridine receptor (DHPR), or more recently, $Ca_v1.2$) [8,9], and ryanodine receptors (RyR2), large multi-functional $Ca^{2+}$ release channels that are crucial for cardiac development and excitation–contraction (EC) coupling (for reviews see [10–15]). However, unlike defects in $Na^+$ and $K^+$ ion handling, cellular $Ca^{2+}$ dysfunction does not arise exclusively from $Ca^{2+}$ channel abnormalities, but also from mutation-linked defects in intra-organellar $Ca^{2+}$ storage (calsequestrin (CSQ), a major $Ca^{2+}$ binding protein of the sarcoplasmic reticulum (SR) [16–18]), $Ca^{2+}$ sequestration (phospholamban (PLB), a regulator of the SR $Ca^{2+}$ ATPase (SERCA) [19,20]) and the altered 'shaping' of cytoplasmic $Ca^{2+}$ signals by cytoplasmic $Ca^{2+}$ binding proteins involved in EC coupling (tropomyosin and troponin [21–23]). Furthermore, alterations in cytoskeletal architecture that disrupt the spatial organisation of $Ca^{2+}$ signalling networks may be highly arrhythmogenic in the absence of any genetic defects in $Ca^{2+}$ handling proteins *per se* [24]. Consequently, the complex physical and functional interplay between $Ca^{2+}$ pumps, channels, stores and exchangers predicts that defects in diverse aspects of cardiomyocyte $Ca^{2+}$ cycling directly contribute to an increased arrhythmogenic propensity.

In this review, we focus on mutations in RyR2 associated with catecholaminergic polymorphic ventricular tachycardia (CPVT), and evaluate recent developments in mutation identification, our understanding of the mechanisms of RyR2 $Ca^{2+}$ release dysfunction and provide an update on the therapeutic potential of RyR2-targeted anti-arrhythmic strategies.

## 2. RyR2 mutations: an etiopathological update

To date, sixty-nine RyR2 mutations have been identified that cause CPVT, a distinct form of early onset stress-induced malignant VT in which affected individuals present with syncopal events and with a distinctive pattern of stress-related, bi-directional VT in the absence of either structural heart disease or a prolonged QT interval [25]. The etiopathology and molecular genetics of RyR2 mutation-linked CPVT (CPVT1) have been reviewed [26–31], but in view of the substantial number of mutations that have recently been reported, a clearer picture of phenotype:genotype correlations, gender bias and patterns of mutation occurrence is beginning to emerge (Table 1).

The gene encoding RyR2 (chromosome 1q42.1–43) is one of the largest and most complex in the human genome (>800 kb, 105 exons) and comprehensive screening strategies are time consuming and expensive. Nevertheless, the detection of novel mutations and the number of RyR2 mutation carriers identified continues apace (Fig. 1). In addition to exhibiting a particularly severe phenotype and a cumulative mortality of 30–50% by 35 years [26,35,52], CPVT1 has been linked with an earlier age of onset than non-genotyped CPVT, and an increased risk of syncope in males [35]. Although some cohorts do exhibit a pronounced sex bias [35,42], analysis of published data reveals no significant gender bias in CPVT1 affected individuals (46% male, 54% females ($n=253$), NSD by chi-square analysis) (Table 1), nor in the male:female incidence of sudden death among screened populations (57.6% males vs. 42.4% females ($n=85$), NSD by chi-square analysis). In contrast, sudden death occurs at a significantly younger age in CPVT1-affected males (19.2±10.6 years vs. 23.6±11.3 years in females, $p=0.036$). The underlying reasons for the significantly increased male lethality are unknown.

Phenotypic manifestation in CPVT1 is extremely heterogeneous and this, together with a highly variable penetrance (that appears to be dependent on the nature of the mutation [36]), results in a significant number of asymptomatic RyR2 mutation carriers. Further phenotypic complexity is suggested by recent reports that distinct mutations in an asymptomatic father (G4662S) and mother (H4762P) resulted in pronounced arrhythmia susceptibility in the progeny [47], in contrast to another 'double' mutant (R176Q and T2504M) [33] in which the individual mutants are pathogenic [36,45]. Furthermore, the combined presence of two commonly occurring polymorphisms, G1885E and G1886S, that occur at an estimated frequency of up to 8% in the normal population is associated with cardiomyopathy [53]. This complex phenotype, compounded by the significant misdiagnosis of patients with RyR2