Cardiomyopathies and sudden cardiac death caused by RyR2 mutations: Are the channels the beginning and the end?☆

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A basic goal of medicine is to cure diseases. Acquired diseases may sometimes be prevented by various methods of exercising, by the “healthy way” of living. Inherited diseases are different; their effective treatment (provided that there is any) requires the recognition of the disease in a sufficiently early stage of life. One – and probably the most promising – way to do so is to establish the link between disorders and genetic alterations. This can be achieved by mapping the mutations corresponding to a given disease, and in the past decade a large number of mutations affecting molecules involved in calcium handling of skeletal and cardiac muscles, including the ryanodine receptor, have been identified [1]. Regarding the cardiac sarcoplasmic reticulum (SR) calcium release channel (RyR2), one of the first published mutations was presented in the pioneering work of Laitinen et al. [2]. In that study, three different RyR2 mutations (P2328S, Q4201R, and V4653F) were associated with familiar polymorphic ventricular tachycardia (FPVT), and each of them was shown to fully co-segregate with the characteristic arrhythmic phenotype. The importance of this kind of knowledge cannot be overestimated, since it makes it possible to recognize a hidden inherited cardiac disorder that exhibits no visible sign of myocardial damage while threatening with close to 30% mortality due to sudden cardiac death (SCD) upon heavy exercise.

The article by Milting et al. in this issue of Cardiovascular Research reports two new single nucleotide polymorphisms (G1885E and G1886S) [3]. In this excellent work, the authors screened the RyR2 gene of 463 healthy volunteers and of 85 patients suffering from arrhythmogenic right ventricular cardiomyopathy (ARVC) and a further 79 having dilated cardiomyopathy (DCM). Surprisingly, an almost equal fraction (85–88%) of the three groups showed the wild-type sequence, indicating that carrying one of the mutations does not necessarily result in clinical symptoms. Only 3% of the ARVC patients were found to carry both mutations (G1885E and G1886S) in a composite heterozygous form. The RyR2 of this genotype exhibited a complex subconductance pattern; one subconductance state had a greatly enhanced open probability resulting in an increased diastolic calcium leak. In addition, the double mutation generated an additional phosphorylation site on the RyR2 that is known to modify its FKBP 12.6 binding affinity. It must be borne in mind, however, that although these mutations were apparently linked to the diseases, the carriers represented only a small fraction of the screened patients. It is not rare that a given mutation is involved in more than one disease, but it is peculiar that a dual mutation on the same protein results in one type of myopathy, while a single mutation results in more than one.

The use of model systems may accelerate the establishment of the link between RyR2 mutations and myocardial diseases, as it was demonstrated for catecholaminergic polymorphic ventricular tachycardia (CPVT) and arrhythmogenic right ventricular dysplasia (ARVD) using mutated RyR2 expressed in HL-1 cardiomyocytes. George et al. reported altered calcium handling induced by three different CPVT-linked mutations (S2246L, N4104K, and R4497C) in the human RyR2 [4]. This information was accentuated by the paper by Lehnart et al.
demonstrating that all FPVT-associated missense mutations in the hRyR2 gene resulted in a leaky cardiac SR calcium release channel [5]. Calcium leak from the SR may generate abnormal calcium homeostasis of cardiac cells leading ultimately to cardiomyopathies combined with life-threatening arrhythmias developing mainly due to afterdepolarizations and reentry. In addition to the classic interpretation based on the increased incidence of delayed afterdepolarizations upon calcium overload, a further possible mechanism may be the calcium-induced suppression of the inward rectifier potassium current, I_{K1} [6], which may facilitate the prevalence of early afterdepolarizations — particularly in patients already treated with class 3 antiarrhythmics. In summary, most of the known RyR2 mutations are likely associated with various types of cardiac arrhythmias elicited by the increased calcium leak through the mutant RyR2 [7].

Since the consequences of some CPTV mutations could be – at least partially – eliminated by administration of the experimental drug K201 (JTV-519), a promising therapeutic strategy seems to emerge. The significant defect of the gain-of-function operation revealed in the study of Lehnart et al. is in line with the hypothesis that the leaky, mutated calcium release channels can be reverted by K201, which restores the gating properties of the channel by enhancing the binding of FKBP 12.6 to the RyR2 [5]. PKA-dependent phosphorylation was shown to decrease the ability of RyR2 to bind FKBP 12.6 (this is how catecholamines can trigger CPVT); however, the exact mechanism of the K201-induced restoration of the RyR2–FKBP 12.6 interaction still remains to be elucidated. Involvement of the RyR2–FKBP 12.6 interaction in arrhythmogenesis was further supported by a cohort study in which 269 patients suffering from long QT syndrome were tested for RyR2 mutations using genomic DNA [8]. In 6.3% of the cases, RyR2 mutation in the FKBP 12.6 binding domain of the channel was verified.

In addition to changes in the RyR2–FKBP 12.6 interaction, altered interdomain interactions have been reported in the case of RyR2 mutations [9]. Using high-resolution confocal microscopy and fluorescence resonance energy transfer techniques, it was demonstrated that some RyR2 mutations resulted in abnormal interdomain interactions upon activation of RyR2, while in resting cells these interdomain interactions remained normal. It was concluded that interdomain interactions among the domains of the RyR2 polypeptide chain play a critical role in the regulation of the channel, and altered domain interactions may cause channel dysfunction in the myopathic heart [10].

Alterations in the RyR2–FKBP 12.6 interaction and interdomain interactions are not exclusive mechanisms by which RyR2 mutation can modify cardiac calcium homeostasis. HEK cells and HL-1 cardiomyocytes expressing mutant C-terminal (Q4201R and I4867M), N-terminal (R176Q and L433P), and central (S2246L and R2474S) regions of the RyR2 molecule were shown to have increased sensitivity to luminal, but not to cytosolic, calcium activation [11]. Interestingly, all of these mutant cells – independently of the site of mutation – displayed an enhanced propensity for store overload-induced calcium release without any evidence for an altered RyR2–FKBP 12.6 interaction, suggesting that although the final consequences of the various RyR2 mutations are uniform (namely, they result in increased cytosolic calcium concentration), the underlying mechanisms may be largely different. In line with this

Fig. 1. Schematic structure of the RyR2 polypeptide. Closed circles represent mutations discussed in the text. The two open circles indicate mutations described recently by Milting et al. [3].
concept, Thomas et al. also found marked differences in cases of various RyR2 mutations [12]. In that report, all of the mutations (L433P, N2386I, and R176Q/T2504M), studied in HEK cells showed profoundly altered calcium handling, including heterogeneous calcium release profiles and elongated intracellular calcium transients; however, one of them (L433P) exhibited a markedly reduced sensitivity to calcium activation.

The few examples mentioned above (and demonstrated in Fig. 1) show that cardiomyopathies based on RyR2 mutations exhibit markedly divergent properties depending on the actual site of the mutation. At the present stage of our knowledge, we have to share the conclusion of Thomas et al. suggesting that myopathies and RyR2 mutations are linked, but in a very complex and largely unknown fashion [13]. The magic stick should pinpoint the governing factor — or more preferably the point of possible correction — of the abnormality, and in that case we have found the specific needle in this particular haystack — at least for the given disease.

References