Editorial

Clinical Implications of Cardiac Ryanodine Receptor/Calcium Release Channel Mutations Linked to Sudden Cardiac Death

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The cardiac ryanodine receptor (RyR2) is the major calcium (Ca^{2+}) release channel on the sarcoplasmic reticulum (SR) in cardiomyocytes. During excitation-contraction, coupling intracellular Ca^{2+} stored in the SR is released via RyR2 to activate muscle contraction. In the heart, excitation-contraction coupling is activated by Ca^{2+} influx via the L-type Ca^{2+} channel that activates RyR2, a process referred to as Ca^{2+}-induced Ca^{2+} release.[1,2] The cardiac muscle RyR2 and its homologue, the skeletal muscle RyR1, are macromolecular complexes that include four ≈565-kDa RyR1 or RyR2, four FKBP12 or FKBP12.6 (12-kDa peptidyl-prolyl isomerases that are required for normal gating of the channels), as well as cAMP-dependent kinase (PKA), phosphatases, and their targeting proteins.[3–5] One key role for the macromolecular signaling complex is to modulate channel function in response to activation of the sympathetic nervous system (ie, the classic "fight-or-flight" stress response).[5,6]

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In the past year, three groups have independently discovered at least 21 mutations in RyR2 (Figure) that are linked to stress-induced sudden cardiac death.[7–9] To date, RyR2 mutations have been associated with 2 forms of sudden cardiac death (SCD): (1) catecholaminergic polymorphic ventricular tachycardia (CPVT) or familial polymorphic ventricular tachycardia (FPVT), and (2) arrhythmogenic right ventricular dysplasia type 2 (ARVD2).

CPVT and FPVT are acronyms for similar, autosomal, dominantly inherited disorders, characterized by adrenergic (exercise or stress)–induced, bidirectional, and polymorphic ventricular tachycardias that cause SCD in the absence of gross structural disease of the myocardium.[10,11] Mortality rates are high, reaching 30% to 50% in patients aged ≤30 years.[12] The original definition of CPVT described children with stress-induced ventricular arrhythmias that are predominantly bidirectional ventricular tachycardias,[10] whereas the FPVT patients have predominantly polymorphic ventricular tachycardia.[8]

Priori et al[7] reported 4 RyR2 missense mutations, 3 of which (S2246L, R2474S, and N4104K) were sporadic and one (R4497C) of which was found in a family with 5 clinically affected mutation carriers. Laitinen et al[8] demonstrated 3 unrelated Finnish FPVT families carrying missense mutations P2328S, Q4201R, and V4653F.

There are at least 6 genetically distinct forms of primarily autosomal, dominantly inherited arrhythmogenic right ventricular dysplasia (ARVD) cardiomyopathies, characterized by progressive degeneration of the right ventricular myocardium, arrhythmias, and SCD.[13,14] Rampazzo et al[15] first mapped ARVD2, which is also characterized by exercise-induced SCD, to chromosome 1q42-q43. Tiso et al[9] reported RyR2 mutations in 4 ARVD2 families; 2 families carried an N2386I mutation, and haplotype analysis suggested that they shared a recent common ancestor. One family had 2 different mutations (R176Q and T2504M) in the same allele, and a fourth family had an L433P missense mutation.

Identification of these SCD-causing mutations has permitted presymptomatic screening in each of these families, which is of great significance in a life-threatening disease like CPVT/FPVT in which the first symptoms are often lethal.

The clinical presentation of patients with RyR2-linked CPVT/FPVT/ARVD2 mutations suggests an association with increased adrenergic activity due to sympathetic nervous system stimulation. Thus, the molecular pathophysiology of SCD in patients with CPVT/FPVT/ARVD2 may be analogous to SCD in patients with heart failure.[16] In failing hearts, a chronic hyperadrenergic state is associated with PKA hyperphosphorylation of RyR2, which depletes the channel of the regulatory subunit FKBP12.6.[5] A diastolic leak of Ca^{2+} due to a hyperactive RyR2 may be one signal that accounts for delayed afterdepolarizations that trigger ventricular tachycardia. Interestingly, 8 of the RyR2 mutations linked to SCD are in the FKBP12.6-binding region of the channel (Figure).

Priori et al[17] provide important new clinical information showing that the ≈50% of families with CPVT who have RyR2 mutations exhibit earlier onset of stress-induced ventricular tachycardia, compared with CPVT individuals without RyR2 mutations. Moreover, males with RyR2 mutations had a higher risk of syncope (relative risk of 4.2). These findings have important implications for the care of patients with CPVT, and the authors recommend the use of prophylactic β-adrenergic receptor blockers in all male children who

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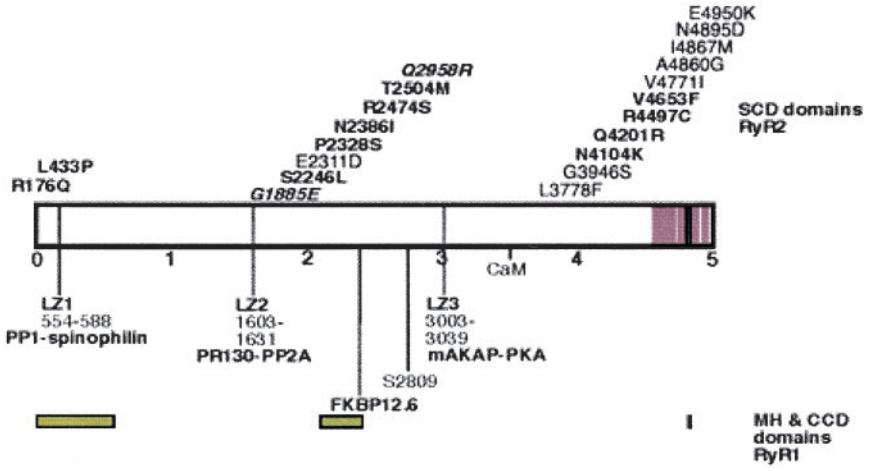

Exercise-induced SCD-linked RyR2 mutations. Locations of SCD mutations in human RyR2 compared with MH/CCD regions of RyR1 and known regulatory domains of the channel. Eleven reported SCD-linked RyR2 mutations[7–9] cluster in three regions homologous to three MH/CCD regions.[30,31] Common polymorphisms are indicated by italics. The location of 3 RyR2 leucine/isoleucine zippers (LZ) that target PP1, PP2A, and PKA to RyR2 are shown,[5,6] as is the FKBP12.6 binding region,[32] and the CaM binding site.[33] The purple lines are the pore region.

are carriers of RyR2 mutations. However, ≈30% of the patients with CPVT required an implantable defibrillator.

One implication of the studies linking RyR2 mutations to stress-induced SCD is that the alterations in structure make the mutant channels hypersensitive to the downstream effectors of the β-adrenergic signaling pathway, namely phosphorylation by PKA. Stress-induced activation of the sympathetic nervous system results in PKA phosphorylation of RyR2, which dissociates FKBP12.6 from the channel and increases the Ca^{2+}-induced activation of the channel. In failing hearts, RyR2 are PKA hyperphosphorylated such that 3 or 4 of the PKA sites in each channel complex are phosphorylated and the channels are depleted of FKBP12.6, resulting in an SR Ca^{2+} "leak." It may be that the RyR2 mutations linked to SCD make the channels more sensitive to activation by PKA phosphorylation in such a way that, under particularly stressful conditions, the mutant channels act like the PKA-hyperphosphorylated channels in failing hearts. The resulting SR Ca^{2+} leak could activate inward, depolarizing currents via the Na^+/Ca^{2+} exchanger, possibly causing delayed afterdepolarizations that are known to trigger fatal ventricular arrhythmias.[18]

RyR2 mutations linked to SCD could alter the PKA phosphorylation modulation of the channel by increasing PKA targeting to the channel or decreasing phosphatase (PP1 and PP2A) targeting to the channel. We have recently shown[5,6] that PKA, PP1, and PP2A are targeted to RyR2 via targeting proteins that bind via leucine/isoleucine zipper motifs in the channel. Interestingly, we recently demonstrated a defect in the leucine/isoleucine zipper-mediated targeting of PKA and PP1 to the potassium channel KCNQ1, linked to exercise-induced SCD in individuals with long-QT syndrome.[19] However, to date none of the SCD-linked RyR2 mutations have been found in sequences that are known to mediate PKA, PP1, or PP2A targeting to RyR2. It is important to emphasize that individuals with CPVT/FPVT/ARVD2-linked RyR2 mutations only manifest symptoms under conditions of sympathetic nervous system activation, so the expectation is that under nonstimulated conditions, these mutant channels would likely have normal biophysical properties.

None of the studies published to date have addressed the biophysical defects attributable to mutations in RyR2. Nevertheless, additional clues about the possible functional effects of the RyR2 SCD mutations come from the observation that the CPVT/FPVT/ARVD2 mutations cluster in 3 regions of the channel, corresponding to malignant hyperthermia (MH) and central core disease (CCD) domains in RyR1. MH and CCD are diseases of skeletal muscle,[20] and their mutations may alter the Ca^{2+}-dependent regulation of RyR1,[21–23] although there have been conflicting reports.[24,25] Single channel studies of mutant RyR1 channels isolated from MH pigs[26] (containing an R615C missense mutation) revealed an increased sensitivity to activation by Ca^{2+} and a decreased sensitivity to inhibition by Mg^{2+}.[27] These alterations in the biophysical properties of the channels could cause an SR Ca^{2+} leak.

In terms of therapeutic approaches for CPVT/FPVT/ARVD2-linked SCD, support for the concept that systemic β-blockers can "protect" the RyR2 channel from the consequences of sympathetic nervous system activation can be derived from our recent study showing that systemic β-blockers can reverse the PKA hyperphosphorylation of RyR2 in failing hearts and restore the normal structure and function of the channel.[28]

It is important to note that Priori et al[17] found individuals with CPVT whose initial symptoms occurred in adulthood, not just in childhood, as previously thought. Patients with CPVT linked to RyR2 mutations were predominantly male and developed symptoms earlier in life than did those without RyR2 mutations, who were predominantly female. Exercise stress testing is currently the best diagnostic tool to identify patients with stress-induced ventricular tachycardia (invasive testing with programmed electrical stimulation and isoprenaline in fusion were not found to be of added diagnostic utility), and early genotyping of all children in families with known RyR2 mutations is warranted because of the high incidence of lethal ventricular arrhythmias that are the first clinical presentations in these individuals.

Genes other than RyR2 have been implicated in catecholamine-induced ventricular tachycardia as well. For example, Eldar and colleagues[29] reported on Bedouin families in Israel that carry an autosomal recessive form of catecholamine- or

exercise-induced polymorphic ventricular tachycardia with mutations in calsequestrin.

The Priori et al study provides novel insights into the clinical manifestations of RyR2 mutations linked to SCD, highlighting the importance of establishing accurate genetic diagnoses in patients with cardiovascular diseases. Sadly, despite nearly a decade of evidence that genetic diagnoses can have an impact on prognosis and help direct therapy in a range of cardiovascular diseases (including hypertrophic cardiomyopathies, long-QT syndrome, Brugada syndrome, and now CPVT), for the most part, clinical facilities required to deliver genetic diagnoses of cardiovascular diseases are inadequate, even in the most highly developed healthcare systems. Moreover, medical education, and in particular, cardiology fellowship training programs, are often not adequately preparing physicians who can provide sophisticated genetic-based clinical care for patients with inherited forms of cardiovascular disease. Thus, the basic science is forging ahead of the capacities of the healthcare system.

Before this gap between knowledge and the delivery of health care widens further, it would be prudent for training programs to incorporate more didactic and clinical training in cardiovascular genetics. Meanwhile, an understanding of the molecular mechanisms by which RyR2 mutations predispose to stress-induced ventricular arrhythmias should emerge from studies of the biophysical properties of the mutant channels and from genetic animal models in which the mutant RyR2 can be studied in vivo. Although insights about the molecular pathogenesis of CPVT/FPVT/ARVD2 should lead to novel therapeutics, the time is already at hand for acquiring genetic diagnoses in appropriate individuals that can improve the care of patients.

References

1. Fabiato A, Fabiato F. Calcium and cardiac excitation-contraction coupling. *Annu Rev Physiol*. 1979;41:473–484.
2. Nabauer M, Callewart G, Cleeman L, et al. Regulation of calcium release is gated by calcium current, not gating charge, in cardiac, myocytes. *Science*. 1989;244:800–803.
3. Marks AR, Tempst P, Hwang KS, et al. Molecular cloning and characterization of the ryanodine receptor/junctional channel complex cDNA from skeletal muscle sarcoplasmic reticulum. *Proc Natl Acad Sci*. 1989; 86:8683–8687.
4. Brillantes AB, Ondrias K, Scott A, et al. Stabilization of calcium release channel (ryanodine receptor) function by FK506-binding protein. *Cell*. 1994;77:513–523.
5. Marx SO, Reiken S, Hisamatsu Y, et al. PKA Phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. *Cell*. 2000;101:365–376.
6. Marx SO, Reiken S, Hisamatsu Y, et al. Phosphorylation-dependent regulation of ryanodine receptors. A novel role for leucine/isoleucine zippers. *J Cell Biol*. 2001;153:699–708.
7. Priori SG, Napolitano C, Tiso N, et al. Mutations in the cardiac ryanodine receptor gene (hRyR2) underlie catecholaminergic polymorphic ventricular tachycardia. *Circulation*. 2001;103:196–200.
8. Laitinen PJ, Brown KM, Piippo K, et al. Mutations of the cardiac ryanodine receptor (RyR2) gene in familial polymorphic ventricular tachycardia. *Circulation*. 2001;103:485–490.
9. Tiso N, Stephan DA, Nava A, et al. Identification of mutations in the cardiac ryanodine receptor gene in families affected with arrhythmogenic right ventricular cardiomyopathy type 2 (ARVD2). *Hum Mol Genet*. 2001;10:189–194.
10. Leenhardt A, Lucet V, Denjoy I, et al. Catecholaminergic polymorphic ventricular tachycardia in children: a 7-year follow-up of 21 patients. *Circulation*. 1995;91:1512–1519.
11. Swan H, Piippo K, Viitasalo M, et al. Arrhythmic disorder mapped to chromosome 1q42-q43 causes malignant polymorphic ventricular tachycardia in structurally normal hearts. *J Am Coll Cardiol*. 1999;34: 2035–2042.
12. Fisher JD, Krikler D, Hallidie-Smith KA. Familial polymorphic ventricular arrhythmias: a quarter century of successful medical treatment based on serial exercise-pharmacologic testing. *J Am Coll Cardiol*. 1999;34: 2015–2022.
13. Marcus FI, Fontaine GH, Guiraudon G, et al. Right ventricular dysplasia: a report of 24 adult cases. *Circulation*. 1982;65:384–398.
14. Fontaine G, Fontaliran F, Frank R. Arrhythmogenic right ventricular cardiomyopathies: clinical forms and main differential diagnoses. *Circulation*. 1998;97:1532–1535.
15. Rampazzo A, Nava A, Erne P, et al. A new locus for arrhythmogenic right ventricular cardiomyopathy (ARVD2) maps to chromosome 1q42-q43. *Hum Mol Genet*. 1995;4:2151–2154.
16. Marks AR. Ryanodine receptors/calcium release channels in heart failure and sudden cardiac death. *J Mol Cell Cardiol*.;2001;33:615–624.
17. Priori S, Napolitano C, Memmi M, et al. Clinical and molecular characterization of patients with catecholaminergic polymorphic ventricular tachycardia. *Circulation*. 2002;106:69–74.
18. Marks AR, Reiken S, Marx SO. Progression of heart failure: is protein kinase a hyperphosphorylation of the ryanodine receptor a contributing factor? *Circulation*. 2002;105:272–275.
19. Marx SO, Kurokawa J, Reiken S, et al. Requirement of a macromolecular signaling complex for beta adrenergic receptor modulation of the KCNQ1-KCNE1 potassium channel. *Science*. 2002;295:496–499.
20. McCarthy TV, Quane KA, Lynch PJ. Ryanodine receptor mutations in malignant hyperthermia and central core disease. *Hum Mutat*. 2000;15: 410–417.
21. Richter M, Schleithoff L, Deufel T, et al. Functional characterization of a distinct ryanodine receptor mutation in human malignant hyperthermia-susceptible muscle. *J Biol Chem*. 1997;272:5256–5260.
22. Lynch PJ, Tong J, Lehane M, et al. A mutation in the transmembrane/luminal domain of the ryanodine receptor is associated with abnormal Ca2+ release channel function and severe central core disease. *Proc Natl Acad Sci U S A*. 1999;96:4164–4169.
23. Tong J, McCarthy TV, MacLennan DH. Measurement of resting cytosolic Ca2+ concentrations and Ca2+ store size in HEK-293 cells transfected with malignant hyperthermia or central core disease mutant Ca2+ release channels. *J Biol Chem*. 1999;274:693–702.
24. Censier K, Urwyler A, Zorzato F, et al. Intracellular calcium homeostasis in human primary muscle cells from malignant hyperthermia-susceptible and normal individuals. Effect of overexpression of recombinant wild-type and Arg163Cys mutated ryanodine receptors. *J Clin Invest*. 1998;101:1233–1242.
25. Avila G, O'Brien JJ, Dirksen RT. Excitation-contraction uncoupling by a human central core disease mutation in the ryanodine receptor. *Proc Natl Acad Sci U S A*. 2001;98:4215–4220.
26. Shomer NH, Louis CF, Fill M, et al. Reconstitution of abnormalities in the malignant hyperthermia-susceptible pig ryanodine receptor. *Am J Physiol*. 1993;264:C125–135.
27. Laver DR, Owen VJ, Junankar PR, et al. Reduced inhibitory effect of Mg2+ on ryanodine receptor-Ca2+ release channels in malignant hyperthermia. *Biophys J*. 1997;73:1913–1924.
28. Reiken S, Gaburjakova M, Gaburjakova J, et al. Beta-adrenergic receptor blockers restore cardiac calcium release channel (ryanodine receptor) structure and function in heart failure. *Circulation*. 2001;104:2843–2848.
29. Lahat H, Pras E, Olender T, et al. A missense mutation in a highly conserved region of CASQ2 is associated with autosomal recessive catecholamine-induced polymorphic ventricular tachycardia in Bedouin families from Israel. *Am J Hum Genet*. 2001;69:1378–1384.
30. Jurkat-Rott K, McCarthy T, Lehmann-Horn F. Genetics and pathogenesis of malignant hyperthermia. *Muscle Nerve*. 2000;23:4–17.
31. MacLennan DH. Ca2+ signalling and muscle disease. *Eur J Biochem*. 2000;267:5291–5297.
32. Gaburjakova M, Gaburjakova J, Reiken S, et al. FKBP12 binding modulates ryanodine receptor channel gating. *J Biol Chem*. 2001;276: 16931–16935.
33. Yamaguchi N, Xin C, Meissner G. Identification of apocalmodulin and Ca2+-calmodulin regulatory domain in skeletal muscle Ca2+ release channel, ryanodine receptor. *J Biol Chem*. 2001;276:22579–22585.

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