Ryanodine Receptor Mutation in Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia: Clinical Implications

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Abstract

This is case of a 40-year-old otherwise healthy and physically active gentleman noted the onset of palpitations while running upstairs the day of admission to the emergency room. On arrival to the emergency room, his heart rate was noted to be 250 beats-per-minute (bpm) and electrocardiogram (ECG) demonstrated ventricular tachycardia (VT). Synchronized electrical cardioversion was performed to sinus bradycardia. He was stabilized and admitted to the hospital. There was demonstration of right ventricular VT with an inferior axis, T-wave inversions in the right pre-cordial leads with a right bundle branch block (RBBB) pattern, epsilon waves, and bidirectional premature ventricular contractions (PVC).

Case Presentation

The patient was treated with medical therapy including beta-blockers for VT. A detailed history and physical exam including family history was unremarkable. There was no evidence of ischemic heart disease by computed tomography contrast coronary angiography and an echocardiogram demonstrated a left-ventricular ejection fraction (LVEF) of 50%. Cardiac magnetic resonance imaging (CMRI) was performed and demonstrated right ventricular regional wall motion abnormalities with dysynchrony and a right ventricular end-diastolic dimension index of greater than 110 ml/m^2. In addition, late gadolinium enhancement was demonstrated in the right ventricular myocardium. The constellation of clinical findings and imaging studies were consistent with a diagnosis of arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D). The patient eventually underwent implantation of an implantable cardioverter-defibrillator (ICD) for secondary prevention of sudden cardiac death. The patient did well and was discharged home on hospital day seven to follow-up in clinic for further management and genetic testing.

Ten days after discharge home, the patient had sudden onset palpitations and lightheadedness and experienced a defibrillation shock. Interrogation of the device revealed appropriate ventricular tachyarrhythmia therapy in the ventricular fibrillation (VF) zone for ventricular tachycardia. He otherwise felt well after the event and his beta-blocker was increased after his clinic visit. Four months later, the patient experienced another defibrillation shock after sudden onset palpitations and lightheadedness. Interrogation of his device demonstrated ventricular tachycardia at 240bpm treated with defibrillation by his device.

Genetic testing demonstrated heterozygous mutations in desmoplakin, junctophilin, and ryanodine receptor genes classified as variants of unknown significance (Table 1).

Discussion

Arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D) is a genetic disorder resulting in a spectrum of clinical presentations ranging from asymptomatic individuals to those with ventricular fibrillation storm, biventricular cardiomyopathy, and sudden cardiac death [1]. The diagnosis of ARVC/D can be difficult when presenting data are inconclusive or other diagnoses are entertained by diagnostic testing. However, in our patient the diagnosis can firmly be made by combining clinical and imaging data. The patient meets two major revised criteria for the diagnosis by MRI findings and the presence of epsilon waves on baseline ECG. In addition, he meets two minor criteria with the presence of right ventricular inferior axis VT and T-wave inversions in the right precordial lead V1-V3 in the presence of complete RBBB [2]. A peculiar finding in this patient is the presence of bidirectional couplet PVCs. Bidirectional ventricular tachycardia has been shown be associated with the diagnosis of catecholaminergic polymorphic ventricular tachycardia (CPVT) [3].

Genetic testing revealed mutational variants in the desmoplakin, junctophilin, and ryanodine receptor (RYR2) gene of unknown significance. Desmoplakin mutations have been described in the ARVC/D population and this particular variant (c.3562T>C) has rarely been detected to our knowledge. Junctophilin mutations have not been described in the literature in the context of genetic cardiomyopathies. This mutation has been demonstrated in 0.1% to 0.2% of person of European descent in the NHLBI exome database [4].
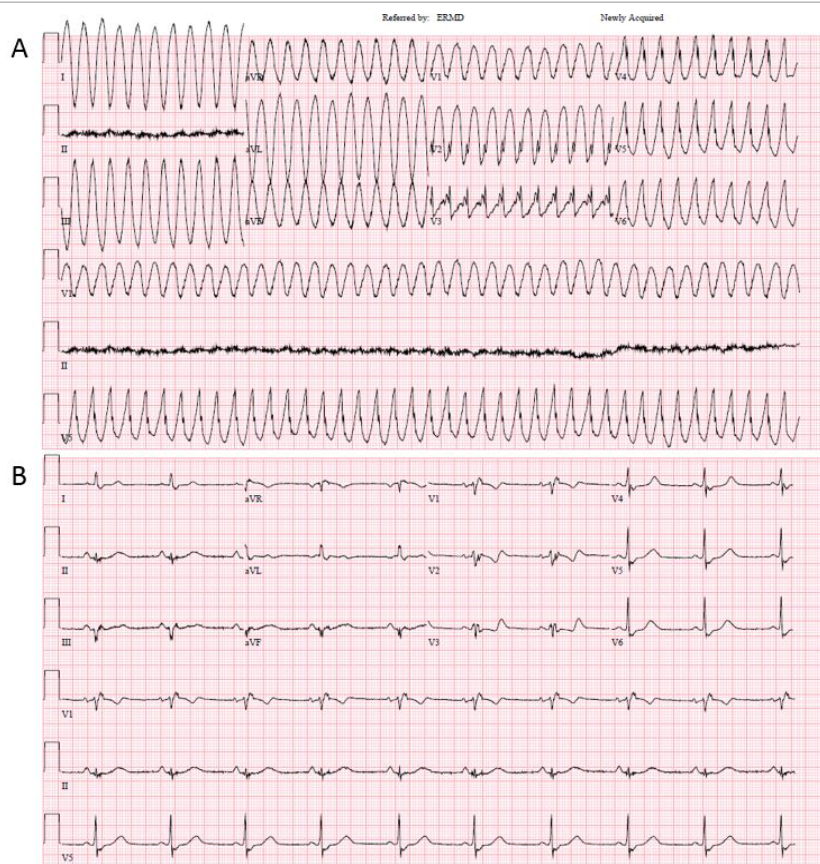
The ryanodine receptors are a class of molecular channels involved in intracellular calcium release in various excitable tissues including the heart. They regulate calcium-induced calcium release from the sarcoplasmic reticulum in the cell and are the major drivers of excitation-contraction coupling in the cardiac myocyte. Calcium release in cardiac ventricular myocytes is associated with increases in inotropy and chronotropy, but also predisposes to ventricular arrhythmias due to enhanced excitation-contraction. In addition,

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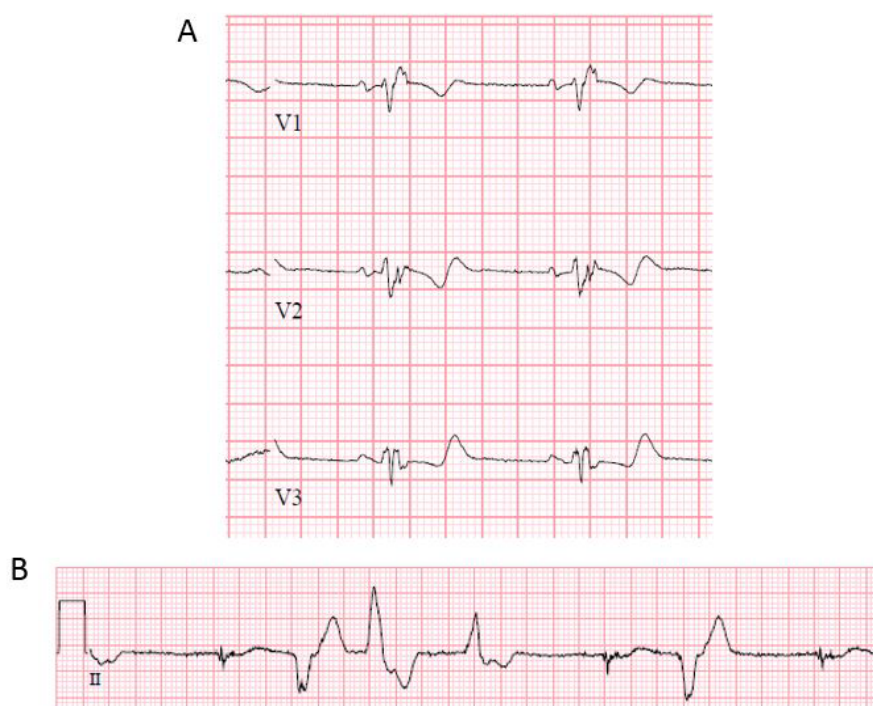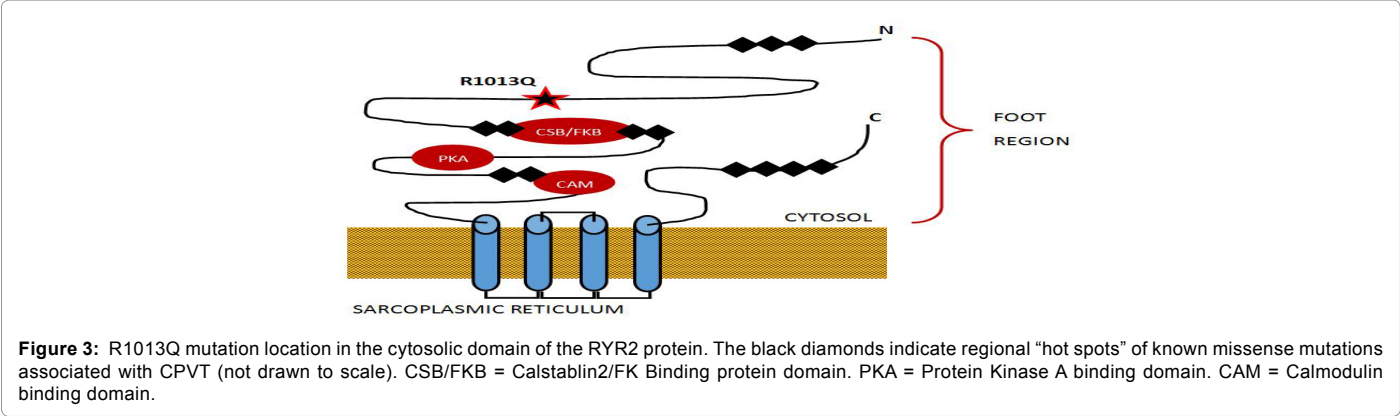
Figure 1: Electrocardiograms. A - Ventricular tachycardia consistent with right ventricular outflow tract origin, B - Baseline ECG with T-wave inversion in a RBBB pattern in pre-cordial leads V1-V3.

Figure 2: A - Magnification of leads V1-V3 with demonstration of epsilon wave and prolongation of the terminal activation of the QRS complex, B - Bi-directional couplet PVCs.

Gene	cDNA	Variant	Zygosity	Classification
DSP	c.3562>C	p.Tyr1188His (Y 1188H)	Heterozygous	Variant of unknown significance
JPH2	c.856 A>G	p.Thr286A1a (T286A)	Heterozygous	Variant of unknown significance
RYR2	c.3038 G>A	p.Arg1013G1n (R 1013Q)	Heterozygous	Variant of unknown significance

Table 1: Results of genetic testing, DSP - desmoplakin gene, JPH2 - junctophilin 2 gene, RYR2 - ryanodine receptor 2 gene.

Figure 3: R1013Q mutation location in the cytosolic domain of the RYR2 protein. The black diamonds indicate regional "hot spots" of known missense mutations associated with CPVT (not drawn to scale). CSB/FKB = Calstablin2/FK Binding protein domain. PKA = Protein Kinase A binding domain. CAM = Calmodulin binding domain.

abnormal regulation of RYR2 is seen in patients with heart failure and missense mutations have been associated with sudden cardiac death. Mutations in the RYR2 result in excess cytosolic calcium in the setting of catecholaminergic stimulation and generates repetitive abnormal excitation of the cardiac myocyte. Repetitive calcium release in the diastolic period during exercise can trigger delayed after-depolarization and promote ventricular arrhythmias [5].

RYR2 mutations have classically been associated with CPVT [6]. Ryanodine receptor mutations have rarely been described in the literature in patients with ARVC/D. In a series of families with the genetic form of ARVC/D with multiple affected family members, Tiso et. al describe four missense mutations in the RYR2, two of which are located in the cytosolic domain of the protein. All four are critical for the regulation of the calcium channel. The authors suggest that in these families with an autosomal dominant inheritance pattern, a gain-of-function mechanism may alter calcium influx during physical activity [7].

The RYR2 mutation found in our patient (R1013Q) has not been described in registries of ARVC/D patients to our knowledge. In addition, this particular ryanodine receptor mutation is not located in the classic "hot spot" regions of the gene for CPVT (Figure 3) and has only been described in one patient with CPVT in a large registry and in an unexplained drowning after molecular autopsy in another series [8,9]. The mutation (c.3038 G >A), which substitutes arginine for glutamine, is a semi-conservative amino acid substitution and may impact molecular structure in a highly conserved residue in the mammalian species. It is located in the cytosolic domain of the protein and may result in instability of the RYR2 and allow calcium leak into the cytosol during membrane depolarization with physical activity (Figure 3).

A variant of unknown significance (VUS), as the name suggests, is a mutation that has not been proven to contribute to disease pathogenesis or has not been well described or studied. Attempts have been made to determine the pathogenicity of VUS mutations including *in silico* studies to predict phenotypic outcomes from mutational variants [10]. However, exome analyses have suggested that VUS maybe be over-represented in the general population and may not be the monogenic cause of certain cardiac channelopathies leading to clinical disease states [11] Although this mutation is deemed a "variant of unknown significance", this case report of the third mutation described in the literature may be more evidence that this mutation plays a role in genetic cardiomyopathies, and may have a clinical impact for novel treatments and family counseling.

The potential therapeutic implications of identifying this mutation in the RYR2 can be extrapolated from the data on patients with CPVT. In a study by Van Der Werf et al. the use of the sodium channel blocker flecainide was associated with a reduction in ventricular tachycardia in patients with CPVT [12]. Molecular studies of flecainide have demonstrated differential effects on the ryanodine receptor however the exact mechanism of action is controversial. *In vitro* evidence suggests that flecainide inhibits the RYR2 during its open state which in turn reduces calcium flux in the diastolic period [13]. However, the physiologic effect of flecainide on the RYR2 has been called into question by Bannister et. al who demonstrated that flecainide had an indirect, sodium and calcium-dependent effect on the RYR2 rather than direct inhibition [14]. Overall, there may be multiple effects of flecainide on the RYR2 [15]. There is sparse data on the use of flecainide in ARVC/D patients.

Conclusion

Previous studies included the use of class I antiarrhythmic drugs with reported rare benefit as compared with class III agents [16]. Ermakov et al. studied a small select population of ARVC/D patient refractory to single antiarrhythmic therapy and clinical benefit with addition of flecainide [17]. At present, there is no clear indication of a monogenic role of the R1013Q mutation in the pathogenesis of ARVD/C. Furthermore, the genetic profile of this patient includes three gene mutations: two gene mutations in structural proteins, one of which has been described in patients with ARVC/D, and the third gene mutation in the RYR2 gene. The relative contributions of each gene mutation to the phenotype of the disease cannot be ascertained. The multiple mutations identified in this patient provide a genetic basis to suggest that combination antiarrhythmic therapy may need to be considered for refractory ventricular arrhythmias, with potential beneficial effects of flecainide for R1013Q mutations. However, further prospective investigations will be needed to guide clinical management of ARVD/C patients with mutations identified in multiple genes.

References

1. Corrado D, Wichter T, Link MS, Hauer R, Marchlinski F, et al. (2015) Treatment of arrhythmogenic right ventricular cardiomyopathy/dysplasia: An international task force consensus statement. Eur Heart J 36: 3227-3237.

2. Marcus FI, McKenna WJ, Sherrill D, Basso C, Bauce B, et al. (2010) Diagnosis of arrhythmogenic right ventricular cardiomyopathy/dysplasia: proposed modification of the Task Force Criteria. Eur Heart J 31: 806-814.

3. Sy RW, Gollob MH, Klein GJ, Yee R, Skanes AC, et al. (2011) Arrhythmia characterization and long-term outcomes in catecholaminergic polymorphic ventricular tachycardia. Heart Rhythm 8: 6.

4. Van der Zwaag PA, Jongbloed JD, Van den Berg MP, Van der Smagt JJ, Jongbloed R, et al. (2009) A genetic variants database for arrhythmogenic right ventricular dysplasia/cardiomyopathy. Hum Mutat 30: 1278-1283.

5. Lehnart SE, Wehrens XH, Kushnir A, Marks RA (2004) Cardiac ryanodine receptor function and regulation in heart disease. Ann N Y Acad Sci 1015: 144-159.

6. Swan H, Piippo K, Viitasalo M, Heikkilä P, Paavonen T, et al. (1999) Arrhythmic disorder mapped to chromosome 1q42-q43 causes malignant polymorphic ventricular tachycardia in structurally normal hearts. J Am Coll Cardiol 34: 2035-2042.

7. Tiso N, Stephan DA, Nava A, Bagattin A, Devaney JM, et al. (2001) Identification of mutations in the cardiac ryanodine receptor gene in families affected with arrhythmogenic right ventricular cardiomyopathy type 2 (ARVD2). Hum Mol Genet 10: 189-194.

8. Medeiros-Domingo A, Bhuiyan ZA, Tester DJ, Hofman N, Bikker H, et al. (2009) The RYR2-encoded ryanodine receptor/calcium release channel in patients diagnosed previously with either catecholaminergic polymorphic ventricular tachycardia or genotype negative, exercise-induced long QT syndrome: a comprehensive open reading frame mutational analysis. J Am Coll Cardiol 54: 2065-2074.

9. Tester DJ, Medeiros-Domingo A, Will ML, Ackerman MJ (2011) Unexplained drownings and the cardiac channelopathies: a molecular autopsy series. Mayo Clin Proc 86: 941-947.

10. Campuzano O, Allegue C, Fernandez A, Iglesias A, Brugada R (2015) Determining the pathogenicity of genetic variants associated with cardiac channelopathies. Sci Rep 5: 7953.

11. Jabbari J, Jabbari R, Nielsen MW, Holst AG, Nielsen JB, et al. (2013) New exome data question the pathogenicity of genetic variants previously associated with catecholaminergic polymorphic ventricular tachycardia. Circ Cardiovasc Genet 6: 481-489.

12. Van der Werf C, Kannankeril PJ, Sacher F, Krahn AD, Viskin S, et al. (2011) Flecainide therapy reduces exercise-induced ventricular arrhythmias in patients with catecholaminergic polymorphic ventricular tachycardia. J Am Coll Cardiol 57: 2244-2254.

13. Hilliard FA, Steele DS, Laver D, Yang Z, Le Marchand SJ, et al. (2010) Flecainide inhibits arrhythmogenic Ca^{2+} waves by open state block of ryanodine receptor Ca^{2+} release channels and reduction of Ca^{2+} spark mass. J Mol Cell Cardiol 48: 293-301.

14. Bannister ML, Thomas NL, Sikkel MB, Mukherjee S, Maxwell C, et al. (2015) The mechanism of flecainide action in CPVT does not involve a direct effect on RyR2. Circ Res 116: 1324-1235.

15. Mehra D, Imtiaz MS, Van Helden DF, Knollmann BC, Laver DR (2014) Multiple modes of ryanodine receptor 2 inhibition by flecainide. Mol Pharmacol 86: 696-706.

16. Wichter T, Borggrefe M, Haverkamp W, Chen X, Breithardt G (1992) Efficacy of antiarrhythmic drugs in patients with arrhythmogenic right ventricular disease. Results in patients with inducible and noninducible ventricular tachycardia. Circulation 86: 29-37.

17. Ermakov S, Hoffmayer KS, Gerstenfeld EP, Scheinman MM (2014) Combination drug therapy for patients with intractable ventricular tachycardia associated with right ventricular cardiomyopathy. Pacing Clin Electrophysiol 37: 90-94.