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Short-coupled polymorphic ventricular tachycardia at rest linked to a novel ryanodine receptor (RyR2) mutation: Leaky RyR2 channels under non-stress conditions

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ABSTRACT

Background: Ryanodine receptor (RyR2) mutations have largely been associated with catecholaminergic polymorphic ventricular tachycardia (PMVT). The role of RyR2 mutations in the pathogenesis of arrhythmias and syncope at rest is unknown. We sought to characterize the clinical and functional characteristics associated with a novel RyR2 mutation found in a mother and daughter with PMVT at rest.

Methods and results: A 31-year-old female with syncope at rest and recurrent short-coupled premature ventricular contractions (PVCs) initiating PMVT was found to be heterozygous for a novel RyR2-H29D mutation. Her mother, who also had syncope at rest and short-coupled PMVT, was found to harbor the same mutation. Human RyR2-H29D mutant channels were generated using site-directed mutagenesis and heterologously expressed in HEK293 cells together with the stabilizing protein calstabin2 (FKPB12.6). Single channel measurements of RyR2-H29D mutant channels and wild type (WT) RyR2 channels were compared at varying concentrations of cytosolic Ca^{2+}. Binding affinities of the RyR2-H29D channels and RyR2-WT channels to calstabin2 were compared. Functional characterization of the RyR2-H29D mutant channel revealed significantly higher open probability and opening frequency at diastolic levels of cytosolic Ca^{2+} under non-stress conditions without protein kinase A treatment. This was associated with a modest depletion of calstabin2 binding under resting conditions.

Conclusions: The RyR2-H29D mutation is associated with a clinical phenotype of short-coupled PMVT at rest. In contrast to catecholaminergic PMVT-associated RyR2 mutations, RyR2-H29D causes a leaky channel at diastolic levels of Ca^{2+} under non-stress conditions. Leaky RyR2 may be an under-recognized mechanism for idiopathic PMVT at rest.

1. Introduction

Polymorphic ventricular tachycardia (PMVT) and ventricular fibrillation (VF) in patients without structural heart disease may account for up to 8% of sudden cardiac death cases [1]. Primary electrophysiological diseases that have been linked to PMVT and VF in patients with structurally normal hearts include: short and long QT syndrome, Brugada syndrome, early repolarization syndrome, catecholaminergic PMVT (CPVT), idiopathic VF and short-coupled torsade de pointes (SC-TdP) [2–7]. In particular, ryanodine receptor (RyR2) mutations have been associated with CPVT, which is characterized by exercise-induced arrhythmias [5]. The role of RyR2 mutations in the pathogenesis of PMVT and VF at rest in patients with structurally normal hearts is unclear.

Here, we report a novel mutation in the cardiac ryanodine receptor (RyR2) gene found in a mother and daughter who had identical presentations of syncope at rest and short-coupled PMVT. After identification of the RyR2 mutation (RyR2-H29D) shared by both patients, we sought to characterize the functional and biochemical consequences of this single amino acid substitution.

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2. Materials and methods

2.1. Clinical characterization

We obtained a complete medical history with emphasis on syncope, near syncope and palpitations in all family members included in this study. Information from echocardiography studies, Holter and event monitors, hospital telemetry, and electrophysiology studies were recorded. Treadmill stress tests were performed using the Cornell protocol [8]. Written informed consent was obtained from family members who agreed to have blood samples obtained for genetic evaluation. This study was approved by the Cornell Institutional Review Board.

2.2. Mutation screening

Genomic DNA was extracted from peripheral blood lymphocytes using standard methods [9]. The RyR2 coding regions were amplified using polymerase chain reaction and analyzed by denaturing high-performance liquid chromatography.

2.3. Functional and biochemical characterization of mutant RyR2 channels

Recombinant mutant channels were generated and expressed in HEK293 cells. The hRyR2-H29D recombinant construct was generated using QuikChange II XL Site-Directed Mutagenesis Kit (*Stratagene*). Single channel measurements were performed to compare RyR2-WT and RyR2-H29D channel properties in planar lipid bilayers. Measurements of calstabin2 binding to immunoprecipitated RyR2-WT and RyR2-H29D channels were performed. A detailed Methods section can be found in the Supplementary Materials.

3. Results

3.1. Clinical phenotype

The proband (III-3 in Fig. 1A) is a 31-year old Indian female who presented to Weill Cornell Medical Center with syncope. While sitting at her desk at work, she had sudden loss of consciousness. She did not recall experiencing any emotional stress prior to the event. She went home and had another episode of syncope at rest. Upon awakening, she called for an emergency medical team. On arrival to the hospital, her ECG revealed sinus rhythm with frequent PVCs (Fig. 2A). Mild early repolarization changes in the inferolateral leads and changes in V_2 that were non-diagnostic for Brugada syndrome were seen. There was no evidence of an abnormal QT interval or signs of arrhythmogenic right ventricular cardiomyopathy. Echocardiography was normal. On telemetry, she had episodes of non-sustained PMVT that were initiated with unifocal PVCs with short coupling intervals of 220–260 ms (Fig. 2B). She was placed on verapamil, but her short-coupled PVCs persisted.

She underwent an electrophysiological study where two PVC morphologies were observed— both with left bundle branch block, left superior axis morphology. The patient was not inducible for ventricular tachycardia or VF with rapid ventricular pacing or up to triple ventricular extrastimuli. Infusion of isoproterenol had no effect on PVC frequency. The two PVCs were mapped to the basal free wall of the right ventricle and ablated. A treadmill exercise test performed after the ablation revealed no PVCs, VT or ischemic changes during exercise. The patient underwent ICD implantation due to concerns for recurrent arrhythmias.

During >7 years of follow-up, the patient had 22 episodes of non-sustained PMVT but no ICD therapies for sustained PMVT. Genetic testing (*Familion*, New Haven, CT) was performed in 2010. The patient was found to be heterozygous for a RyR2-H29D (85 C>G) mutation, which was not seen in a control population of 400 patients. In addition, this RyR2 variant was not present in the Single Nucleotide Polymorphism (dbSNP), 1000 Genomes, or NHLBI Exome Sequencing Project (ESP) databases. Screening for pathogenic mutations for long QT syndrome, Brugada syndrome and early repolarization syndrome (*Familion*, New Haven, CT) was also negative. Full sequence analysis of RyR2 gene exons was performed and the presence of the RyR2-H29D mutation was confirmed with no other mutations detected elsewhere (Fig. 1B).

The proband's mother (II-2 in Fig. 1A) presented in June 1979 at the age of 41 with palpitations and lightheadedness. Her baseline ECG revealed borderline J point elevation in the inferior leads (Fig. 3A). She was noted on monitoring to have ventricular bigeminy and was placed on procainamide. However, she had recurrent syncope at rest. In one episode, the patient experienced syncope while sitting in bed talking to her children. On November 1979, she had an episode of syncope at work and was brought to the hospital. She was found to be in ventricular bigeminy with very short coupling intervals (200 ms) leading to sustained PMVT (Fig. 3B). Cardiac catheterization revealed normal coronary arteries. Control of sustained ventricular arrhythmias was achieved with procainamide and quinidine but due to side effects, she was switched to sotalol. She presented in 2007 with symptomatic sinus bradycardia and a dual chamber pacemaker was implanted. Review of pacemaker diagnostics over >5 years of follow-up revealed no sustained arrhythmias. A genetic screen for the RyR2-H29D mutation in this patient was positive. The patient had a history of gastric cancer and died at the age of 74 of non-cardiac reasons. In both the proband and the proband's mother's cases, cardiac MRI scans were not performed prior to cardiac device implantation. Neither patient was ever found to have signs of arrhythmogenic right ventricular cardiomyopathy by echocardiographic or ECG surveillance over long term follow-up.

The proband's sister (III-1 in Fig. 1A) had a history of syncope without prodrome while opening a door to her home. She had a history of palpitations for several years. She had a normal ECG, echocardiogram and negative treadmill study. A 14-day mobile cardiac outpatient telemetry monitor revealed symptomatic ventricular couplet with a coupling interval of 380 ms but no VT. A genetic screen for the RyR2-H29D mutation in this patient was positive. The proband's brother (III-2 in Fig. 1A) had a remote history of two episodes of syncope as a teenager associated with dizziness and diaphoresis while standing which was felt to be consistent with vasovagal syncope. He denied any history of palpitations. He had a normal ECG, echocardiogram and treadmill study. A 24-hour Holter monitor revealed no ventricular ectopy. His screen for the RyR2-H29D mutation was negative (Fig. 1C). Analysis of RyR2 sequence alignment indicates that the RyR2-H29D mutation is near the amino terminal hot-spot region of RyR2 (Fig. 1D).

3.2. Functional characterization of short coupled TdP-linked RyR2 mutation

In CPVT, single-point mutations in RyR2 can alter the biophysical properties of RyR2 channel, in particular its sensitivity to activation by cytosolic Ca^{2+} under conditions of PKA phosphorylation [10–13]. Since our patients did not have a CPVT phenotype given their arrhythmias and syncope at rest, we postulated that the RyR2-H29D mutation alters RyR2 function under resting conditions. To evaluate the functional consequences of the RyR2-H29D mutation, we examined its single-channel properties in the presence of calstabin2 and compared them to the RyR2-WT channels using a planar lipid bilayer model. Over a range of cytosolic (*cis*) Ca^{2+} concentrations from 150 nmol/L (diastolic level) up to 10 μmol/L (systolic level), we evaluated the single-channel open probability, mean open and close times, and frequency of openings.

Under basal conditions with no PKA phosphorylation and at diastolic cytosolic Ca^{2+} concentrations of 150 nmol/L, RyR2-H29D mutant channels exhibited a significantly higher open probability (Po) when compared to WT channels (mean Po of 0.071 ± S.E.M. of 0.017 (n = 9) for RyR2-H29D vs, mean Po of 0.009 ± 0.001 (n = 5) for RyR2-WT; p = 0.021; Figs. 4A–B and 5A). While the mean open times (To) and close times (Tc) were similar (Online Fig. 1), the frequency of opening (Fo) was significantly higher at 150 nmol/L in RyR2-H29D channels than when compared to RyR2-WT channels (mean Fo of 7551 ± 1549 events/min (n = 8) for RyR2-H29D vs. mean Fo of 988 ± 139 events/min (n = 5) for RyR2-WT, p = 0.007; Figs. 4A–B and 5B). Similarly, at cytosolic Ca^{2+} concentrations of 350 nmol/L, RyR2-H29D mutant channels exhibited a significantly higher Po (mean Po of 0.074 ± 0.019 (n = 9) for RyR2-H29D vs. mean Po of 0.017 ± 0.005 (n = 5) for RyR2-WT, p = 0.047; Figs. 4A–B and 5A) as well as a significantly higher Fo than that of WT channels (mean Fo of 7179 ± 1380 events/