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Brief Communication

Identification of a novel mutation V2321M of the cardiac ryanodine receptor gene of sudden unexplained death and a phenotypic study of the gene mutations

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Abstract

Mutations of the cardiac ryanodine receptor (RyR2) gene cause catecholaminergic polymorphic ventricular tachycardia, which sometimes results in a finding of sudden unexplained death (SUD) at autopsy. We found a novel mutation (V2321M) in exon 46 of the RyR2 gene in a SUD case. V2321M was localized in a highly conservative site of the RyR2 gene, but was not found in 400 reference alleles. We previously reported two SUD cases with R420W mutations in exon 14 of the RyR2 gene. We examined possible phenotypic characteristics of all three of these cases of SUD with the RyR2 gene mutations. All cases displayed mesenteric lymph node hypertrophy as well as tendencies for aortic narrowing. By contrast, only one of the 14 SUD cases without RyR2 mutations displayed these phenotypes. This study supports the concept that postmortem genetic testing of RyR2 mutations should be considered in autopsy examinations of SUD cases. It also raises the possibility that some cases with RyR2 mutations may display phenotypic changes in lymphoid and cardiovascular organs.

Keywords: Arrhythmia; Molecular autopsy; RyR2; Sudden cardiac death; Status thymicolymphaticus

1. Introduction

Forensic pathologists sometimes encounter sudden death cases in apparently healthy young people with no abnormal findings at autopsy. Such cases are usually defined as sudden unexplained death (SUD) [1].

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an arrhythmogenic disease characterized by stress- or exercise-induced ventricular arrhythmia, syncope, or early sudden death. No morphological abnormalities have been reported in hearts from CPVT patients [2,3]. The cardiac ryanodine receptor (RyR2) gene has been identified as a gene responsible for CPVT [2] and for arrhythmogenic right ventricular dysplasia type 2 (ARVD2) [4]. The RyR2 gene, which consists of 105 exons, encodes the cardiac Ca^{2+} release channel, which is localized across the sarcoplasmic reticulum of cardiomyocytes. The channel regulates intracellular Ca^{2+} concentration and excitation–contraction coupling in cardiac muscles [5]. Postmortem genetic screenings reveal that RyR2 mutations are identified in some SUD cases [6–8].

We previously reported two SUD cases with R420W mutations of the RyR2 gene found at autopsy [7]. Interestingly, both cases had similar morphological features in their cardiovascular and lymphoid organs. They displayed mild fatty infiltration to the heart, narrowing of the aorta, and hypertrophy of mesenteric lymph nodes. One case also had enlarged thymus and tonsils. In this study, we report a novel missense mutation of the RyR2 gene in a SUD case, and also study possible phenotypic characteristics of RyR2 gene mutations.

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2. Materials and methods

2.1. SUD cases examined

Seventeen SUD cases, 13 males and 4 females aged from 12 to 42 years, autopsied in our department over a 9-year period were examined. SUD was defined as a sudden, unexpected and unexplained death determined after the conclusion of a medicolegal autopsy including microscopic evaluations.

2.2. Mutational analysis

We performed mutational screening of three hot spots including exons 8–15, 44–47, 49 and 83–105 in the RyR2 gene using a HR-1 High Resolution Melter (Idaho Technology Inc, Salt Lake, UT, USA). The previously reported missense mutations were located in the three regions; in N-terminal (exons 8–15), calstabin 2 binding (exons 44–47 and 49), and transmembrane region (exons 83–105) [14]. Mutation discovery was performed by high resolution melt methods and then confirmed by DNA sequencing. Primer sequences used for the analysis were as previously described [4]. DNA was sequenced using an ABI 310 DNA sequencer (PE Applied Biosystems, Foster City, CA, USA). Isolation and analysis of the DNA of the cases examined were approved by the Ethical Committee for Research of the Human Genome at Osaka Medical College.

3. Case report

A 22-year-old apparently healthy female collapsed during emotional stress and died suddenly. The victim was 156 cm in height, and weighed 58 kg. Her heart weighed 230 g (the average ± SD weight of hearts of age and sex-matched Japanese controls is recorded as 231 ± 35.1 g) [9]. No apparent morphological abnormalities were observed in the deceased's heart (Fig. 1). A number of lymph nodes in the mesentery were recorded as showing enlargement. The spleen weighed 130 g (average ± SD is 99 ± 38.8 g) [9]. The widths of the aorta at the beginning, diaphragm and ending were 5.2, 3.1 and 2.7 cm, respectively (average ± SD widths are 5.4 ± 0.46, 3.5 ± 0.30 and 2.8 ± 0.31 cm, respectively) [9]. The adrenal glands weighed 4.9 g for the left and 4.7 g for the right (average ± SD is 5.9 ± 1.92 for left and 5.5 ± 1.61 for right) [9]. Thymic weight was not recorded.

4. Results

A heterozygous missense mutation (V2321M) was identified in exon 46 of the RyR2 gene (Fig. 2). The RyR2 mutation we found was located in one of the three hot spot

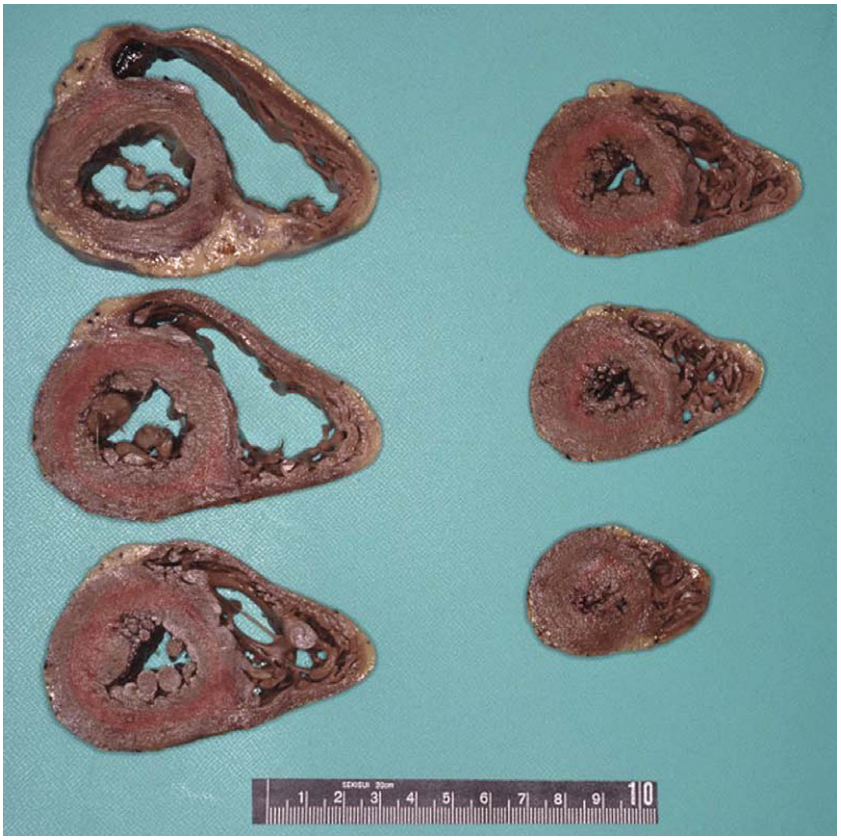

Fig. 1. Transverse sections of the deceased's formalin-fixed heart.