Postmortem Molecular Screening for Cardiac Ryanodine Receptor Type 2 Mutations in Sudden Unexplained Death —— R420W Mutated Case With Characteristics of Status Thymico-Lymphaticus ——

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Background Mutations of the cardiac ryanodine receptor type 2 (RyR2) gene are known to cause effort-induced polymorphic ventricular arrhythmia, syncope and sudden death.

Methods and Results The possible mutations in the RyR2 gene were examined in 18 autopsy cases of sudden unexplained death (SUD). Two cases were found to have the heterozygous missense mutation in exon 14 (nucleotide change C1258T, coding effect R420W). Both cases showed mild fatty infiltration of the right ventricular apex. Interestingly, 1 case showed an enlarged thymus with accompanying hypertrophy of the tonsils and mesenteric lymph nodes. In addition, a narrowing of the aorta was observed in this case. These phenotypic characteristics are consistent with status thymico-lymphaticus, which combines sudden death with an enlargement of lymphoid organs and hypoplasia of the cardiovascular system. The second case also displayed some characteristics of status thymico-lymphaticus.

Conclusion The R420W mutation has already been reported in families with juvenile sudden death and may be causative of sudden death in our cases. Postmortem molecular screening of the RyR2 gene could be useful for investigation for cause of death in SUD. The possible association of the RyR2 mutation with status thymico-lymphaticus is discussed. (Circ J 2006; 70: 1402–1406)

Key Words: Arrhythmia; Autopsy; Calcium; Sudden death; Thymus

Sudden unexplained death (SUD) is defined as sudden death in apparently healthy humans, children in most cases, with no structural abnormalities found at autopsy.1 Fatal arrhythmia has been suggested as being involved in the etiology of SUD.2–4 Recent progress in post-mortem molecular analysis has revealed that mutations of the cardiac ryanodine receptor type 2 (RyR2) gene are involved in some cases of SUD.5

The RyR2 gene, encompassing 105 exons, encodes a Ca2+ channel, which is localized across the sarcoplasmic reticulum (SR) membrane of cardiomyocytes. The channel releases Ca2+ from SR stores into the cytoplasm in response to action potentials, which regulates intracellular Ca2+ concentration and excitation–contraction coupling in cardiac muscle.6

Mutations in the RyR2 gene have been demonstrated in catecholaminergic polymorphic ventricular tachycardia (CPVT), a heritable arrhythmia syndrome that typically manifests in exertional syncope or sudden death.7–9 RyR2 mutations have also been reported to lead to arrhythmogenic right ventricular dysplasia type 2 (ARVD).10 ARVD is characterized by fatty infiltration and fibrosis of the myocardium, resulting in electric instability and risk of fatal ventricular arrhythmias. ARVD2 is clinically different from other forms of ARVD due to the presence of effort-induced ventricular arrhythmia.11 CPVT and ARVD2 have been suggested as corresponding to different degrees of phenotypic expression of the same disease.5,12 Thus, RyR2 mutations are specifically linked to effort-induced polymorphic ventricular arrhythmia, syncope and sudden death, either in patients with a normal heart or in those with ARVD.8,10,13

In the present study we performed postmortem molecular screening for possible mutations of the RyR2 gene in 18 SUD cases, and found 2 cases with the same mutation, which had been previously reported in families with juvenile sudden death. In addition, based on the autopsy findings, we discuss the possible association of the RyR2 gene mutation with status thymico-lymphaticus, which is a condition involving constitutional anomalies such as hypertrophy of lymphoid organs and hypoplasia of the cardiovascular system.14–17

Methods

Examined Cases

We examined 18 SUD autopsy cases from a 9-year period for the present study: 13 males and 5 females, with an age range from 2 to 42 years old. SUD was defined as a sudden, unexpected and unexplained death. In all cases, the autopsy was carried out within 36h of death. The cause of

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death could not be determined by gross and microscopic analysis.

**Mutational Analysis of RyR2**

DNA was extracted from autopsy blood samples using standard phenol-chloroform procedures. A targeted mutational analysis of 24 exons of the RyR2 gene (3, 8, 14, 15, 44–47, 49, 83, 87–91, 93, 94, 97, 100–105) was performed on genomic DNA using a HR-1 High Resolution Melter (Idaho Technology Inc, Salt Lake, UT, USA), and DNA was sequenced using an ABI 310 DNA sequencer (PE Applied Biosystems, Foster City, CA, USA). The polymerase chain reactions were performed using previously published primers. Isolation and analysis of the DNA of the examined cases were approved by the ethical committee for research of the human genome at Osaka Medical College.

**Results**

The RyR2 missense mutation, a change from C to T at nucleotide 1258, was detected in 2 cases. It was predicted to result in a change of arginine to tryptophan at position 420 (G420W) (Fig 1). Both cases were heterozygous for the G420W substitution.

Case 1 was a 13-year-old male who collapsed and died suddenly at gymnastic class. The victim was 161 cm in height, and weighed 45 kg. The heart weighed 239.4 g (the average ± SD weight of the hearts of age and sex-matched Japanese controls is recorded as 219±42.3 g). The right ventricular trabeculae were obvious, and the right ventricular wall was thin (Fig 2a). Microscopic examination showed mild fatty infiltration at the right ventricular apex (Fig 2b). The lungs were highly congested (left, 759.4 g; right, 810.9 g). The spleen weighed 155.5 g (the average ± SD weight was 93±29.0 g). The left tonsil was enlarged (Fig 3a), and gross examination of the lymph nodes in the mesentery showed enlargement (Fig 3b). In addition, the thymus was enlarged to 74.9 g (the average ± SD weight was 47.8±22.3 g) (Fig 4a). Microscopic examination revealed that the thymus was composed of abundant parenchyma and a small amount of adipose tissue (Fig 4b). The widths of the aorta at the beginning, diaphragm and ending were 4.5, 3.2 and 2.5 cm, respectively (the average ± SD widths...
were 4.9±0.38, 3.1±0.12 and 2.5±0.12 cm, respectively).

Case 2 was a 33-year-old female who collapsed and died suddenly while escaping from a dog by bicycle. The victim was 159 cm in height, and weighed 45 kg. The heart weighed 235 g (the average ± SD weight was 254±39.6 g) (Fig 5a). Mild fatty infiltration was observed at the right ventricular apex using microscopic analysis (Fig 5b). The left and right lungs weighed 330 g (the average ± SD weight was 377±146.6 g) and 400 g (431±155.4 g), respectively (Fig 5). The spleen weighed 110 g (the average ± SD weight was 96±37.7 g). The weight of the left and right adrenal glands was 2.8 g (the average ± SD weight was 5.4±1.78 g) and 2.7 g (5.1±1.68 g), respectively. Unfortunately, the thymic weight was not recorded. The width of the aorta at the beginning, diaphragm and ending was 5.2, 3.3 and 2.8 cm, respectively (the average ± SD widths were 5.7±0.59, 3.8±0.32 and 3.0±0.29 cm, respectively).

Discussion

We found the heterozygous R420W mutation in 2 cases of SUD. Both cases involved sudden death during physical exertion. Case 2 might have also involved emotional stress. The R420W mutation was already found at postmortem screening and not in the 400 reference alleles. Additionally, the mutation was reported in 2 families with juvenile sudden death. These findings indicate that the R420W mutation is pathogenic and contributed to the cause of death in Cases 1 and 2 presented here.

Both cases displayed mild fatty infiltration at the right ventricular apex. Previous reports also documented that 2 of the 3 autopsied cases with the R420W mutation showed mild fatty infiltration at the right ventricular apex. A mild fatty infiltration in the right ventricle, particularly at the apex, may be a common feature of the R420W mutation. Because effort-induced sudden death with fatty degeneration of the right ventricle is a characteristic of ARVD, the victims in the cases discussed here may have been unrecognized carriers of ARVD.

Interestingly, Case 1 showed typical characteristics of so-called status thymico-lymphaticus. This condition involves a combination of constitutional anomalies: hyperplasia of lymphoid organs such as the thymus, spleen and lymph nodes, and hypoplasia of the cardiovascular system with narrowing of the aorta. The condition is sometimes terminated by sudden death usually in children. Fatty degeneration of the heart, which was observed in both Case 1 and 2, has also been described as one of the characteristics.
of status thymico-lymphaticus.\(^7\)

Case 1 showed enlargement of the thymus, tonsils and mesenteric lymph nodes. In addition, the heart was hypoplastic, and the width of the aorta was 4.5 cm at the beginning (the average ± SD was 4.9 ± 0.38 cm).\(^9\) Case 2 also showed mild enlargement of the mesenteric lymph nodes (data not shown). In addition, the widths of the aorta were decreased. Thus, the cases presented here displaying the R420W mutation, especially Case 1, had particular features of status thymico-lymphaticus.

The possibility must be considered that the R420W mutation is essentially independent of the status thymico-lymphaticus features in the cases presented here. However, it is also possible that the mutation is at least involved in the observed hypertrophy of lymphoid organs. Interestingly, the RyR2-encoded Ca\(^{2+}\) channel is expressed not only in the cardiac muscle, but also in lymphoid organs including the thymus, and is suggested that it could be involved in the regulation of intracellular Ca\(^{2+}\) concentration in these non-cardiac cells.\(^22\) Because intracellular Ca\(^{2+}\) is known to play important roles in positive and negative selection of thymocytes, an altered intracellular Ca\(^{2+}\) concentration by RyR2 mutations might cause an accumulation of abnormally differentiated thymocytes in lymphoid organs.\(^22\) In such a case, the RyR2 mutation would result in 2 apparently separate phenomena, which are the sudden death due to fatal arrhythmias and the hypertrophy of lymphoid organs. Since the molecular mechanism for status thymico-lymphaticus has not been suggested so far, RyR2 mutations may be a clue towards understanding its molecular basis. Further investigation is necessary to explore the possible involvement of the RyR2 mutation in phenotypic changes of status thymico-lymphaticus.

Recent progress in postmortem molecular analysis has revealed that long QT syndrome is involved in some cases of SUD.\(^2\) We have also examined the possible mutations of genes responsible for long QT syndrome, and found 1 case possessing a novel mutation of KCNQ1, which encodes a cardiac voltage-dependent K\(^+\) channel (unpublished data). Whether the mutation was really involved in the cause of death in the victim is still being investigated.

In conclusion, the successful identification of the pathogenic RyR2 mutation in SUD cases presented here indicates the usefulness of postmortem screening for RyR2 gene defects in investigation of the cause of death. The screening may also prevent living family members from suffering possible sudden death by appropriate medication or lifestyle changes.\(^25\) \(^29\) We have also discussed the possibility that the RyR2 mutation may be associated with status thymico-lymphaticus.

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References

