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【 ORIGINAL ARTICLE 】

Bradycardia Is a Specific Phenotype of Catecholaminergic Polymorphic Ventricular Tachycardia Induced by *RYR2* Mutations

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Abstract:

Objective Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a lethal inherited disease characterized by ventricular arrhythmias induced by physical exercise or emotional stress. The major cause of CPVT is mutations in *RYR2*, which encodes the cardiac ryanodine receptor channel. Recent advances in sequencing technology have yielded incidental findings of *RYR2* variants in other cardiac diseases. Analyzing the characteristics of *RYR2* variants related to CPVT will be useful for differentiation from those related to other cardiac diseases. We examined the phenotypic characteristics of patients with *RYR2* variants.

Methods Seventy-nine probands carrying *RYR2* variants whose diagnoses were either CPVT (n=68) or long QT syndrome (LQTS; n=11) were enrolled. We compared the characteristics of the electrocardiogram (ECG) and the location of the *RYR2* mutations-N-terminal (NT), central region (CR) or C-terminal (CT)-between the two patient groups.

Results Using the ECGs available from 53 probands before β-blocker therapies, we analyzed the heart rates (HRs). CPVT probands showed bradycardia more frequently (25/44; 57%) than LQTS probands (1/9; 11%; p=0.024). In CPVT patients, 20 mutations were located in NT, 25 in CR and 23 in CT. In LQTS patients, 5 mutations were located in NT, 2 in CR and 4 in CT. There were no significant differences in the locations of the *RYR2* mutations between the phenotypes.

Conclusion Bradycardia was highly correlated with the phenotype of CPVT. When a clinically-diagnosed LQTS patient with bradycardia carries an *RYR2* mutation, we should be careful to avoid making a misdiagnosis, as the patient may actually have CPVT.

Key words: catecholaminergic polymorphic ventricular tachycardia, long QT syndrome, bradycardia, *RYR2* mutation

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Introduction

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a lethal inherited primary electrical disease characterized by bidirectional or polymorphic ventricular arrhythmias caused by exercise or emotional stress, particularly in young patients. The diagnosis includes the absence of the other primary electrical disease, such as long QT syndrome (LQTS) (1). CPVT shares clinical features with LQTS in terms of causing faintness or sudden death by exercise or emotional stress. It is therefore often difficult to make differential diagnosis between these two clinical situations (2, 3).

Pathogenic *RYR2* mutations were identified in nearly 60% of patients with CPVT (4). The cardiac ryanodine receptor channel (RyR2) encoded by *RYR2* is a calcium release channel localized on the sarcoplasmic reticulum (SR) membrane. Gain-of-function type *RYR2* mutations cause abnormal Ca^{2+} leak from the SR, resulting in delayed depolarization and

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Table 1. Definition of Bradycardia in This Study.

Age (years)	Heart rate (bpm)
0	<100
1-3	<95
4-5	<75
6-8	<65
≥ 9	<60

ventricular arrhythmias observed in CPVT (1, 5). Mutations in *RYR2* are causative for inherited primary arrhythmia syndromes (IPASs), such as CPVT (1, 5), LQTS (6), short-coupled polymorphic ventricular tachycardia (7, 8) and idiopathic ventricular fibrillation (9).

One issue regarding the genetic screening of *RYR2* is associated with its large size, as the gene consists of 105 exons. The recent clinical introduction of next-generation sequencing (NGS) (10) has allowed us to detect disease-causing mutations not only in *RYR2* but in other candidate genes for IPASs. Using a targeted panel gene analysis with NGS, we extensively identified *RYR2* variants in our cohort of clinically-diagnosed CPVT (n=68) and clinically-diagnosed LQTS (n=11) probands.

Therefore, the present study aimed (1) to examine the phenotypic characteristics of carriers with *RYR2* variants and (2) to clarify the relationships among mutation locations in *RYR2*. These data will offer important insights into the contribution of *RYR2* mutations to IPASs.

Materials and Methods

Patients

Seventy-nine probands carrying *RYR2* variants whose diagnoses were either CPVT (n=68) or LQTS (n=11) were enrolled. Table 1 shows the demographic characteristics of the study population. The diagnosis of CPVT or LQTS was made based on clinical symptoms, electrocardiogram (ECG) findings and modified Schwartz scores (2, 11). When a patient had a Schwartz score of ≥3.5 and showed neither bidirectional ventricular tachycardia (bVT) nor polymorphic ventricular tachycardia (pVT) on ECG, we diagnosed the patient with LQTS. In contrast, when a patient had a Schwartz score of <3.5 or had a Schwartz score of ≥3.5 but showed bVT or pVT on ECG, we diagnosed the patient with CPVT.

We excluded patients being treated with β-blockers from the analysis of the heart rate. Because the normal range of the heart rate varies with age, bradycardia was defined as follows: in children aged <9 years, a heart rate below the second percentile of the established age- and sex-appropriate norms; in adults or children ≥ 9 years, <60 beats/min on resting ECG without taking β-blockers (12, 13) (Table 1). We compared the ECG findings, such as the heart rate and QTc interval, between patients with each disease.

Genetic analyses

The protocol for the genetic analyses was approved by our Institutional Ethics Committee and carried out under the guidance of this committee. All patients and their guardians provided their written informed consent before undergoing a genetic analysis. Genomic DNA was isolated from peripheral white blood cells. *RYR2* variants were screened by the Sanger method using an ABI PRISM-3130 (Applied Biosystems, Foster City, USA) or by targeted gene-sequencing methods using MiSeq (Illumina, San Diego, USA) and confirmed by the Sanger method. NGS screening was also used to search for variants of LQTS-related genes. We excluded from the analysis variants for which the minor allele frequencies (MAFs) in ethnicity-matched controls were greater than 0.005.

All variants were evaluated by several *in silico* predicting software programs: Polymorphism Phenotyping (PolyPhen)-2 (http://genetics.bwh.harvard.edu/pph2/), SIFT (http://sift.jcvi.org/), PROVEAN (http://provean.jcvi.org/index.php) and CADD (http://cadd.gs.washington.edu/score). Scores for each variant are summarized in the Supplementary material. When the variants were reported in the ExAC database (http://exac.broadinstitute.org/), dbSNP database (https://www.ncbi.nlm.nih.gov/snp/) or in the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/), we added the information to the Supplementary material.

RyR2 mutation locations

According to the previous report (14), we categorized the *RYR2* mutation sites as N-terminal (NT: 1-2177), central region (CR: 2178-4075) and C-terminal (CT: 4076-4959).

Statistical analyses

All statistical analyses were conducted using the Stata version 14.0 software program (LightStone, Tokyo, Japan). Continuous variables with normal distribution are shown as the mean ± standard deviation (SD), and variables without a normal distribution are shown as the median [interquartile range (IQR)]. Levene's tests were conducted to assess the equality of variance for comparable groups. Differences between groups were evaluated by Fisher's exact test, Student's *t*-test and the Mann-Whitney U test when necessary. All p values <0.05 were considered statistically significant.

Results

Clinical characteristics of the study population

The demographic characteristics of the study patients are summarized in Table 2. The clinical features of each patient and variant information are summarized in the Supplementary material. Forty-three (54.4%) patients were men, of whom 36 had CPVT and 7 LQTS. The mean age at the time of the genetic analysis showed no significant differences between CPVT and LQTS (p=0.51). However, pa-

Table 2. Demographic Characteristics of the Study Population.

	CPVT	LQTS	p value
Number	68	11	
Male	36 (53%)	7 (64%)	0.75#
Age (IQR)	13.0 (10.0-16.3)	16.0 (8.5-32.0)	0.51*
QTc (IQR)	411.5 (392.8-433.3)	480.0 (460.0-515.0)	<0.001*
Schwartz score	2.3±0.9	4.7±1.5	<0.001**
Patients with CPA	29 (43%)	3 (27%)	0.51#

Values for Age and QTc are represented by median (IQR: inter-quartile range).
Values for Schwartz score is mean±SD.
CPA: cardiopulmonary arrest
Fisher's exact test, *Mann-Whitney U test, **Student t-test

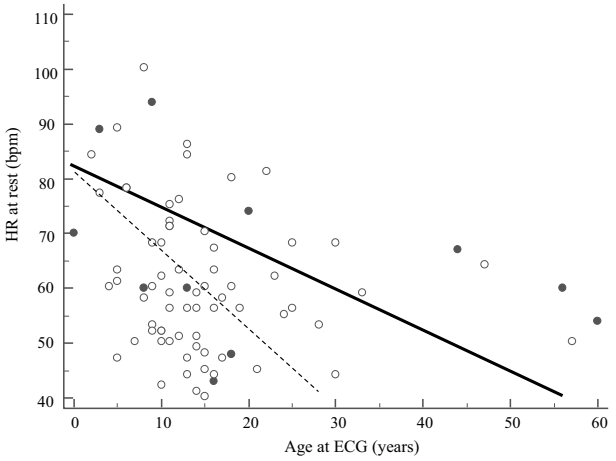

Figure 1. Relationship between the HR at rest and the age of ECG recordings. The HRs in CPVT patients (open circle) were lower than those of LQTS (filled circle). Open circles were fitted to the dotted line and filled circles were to the black line.

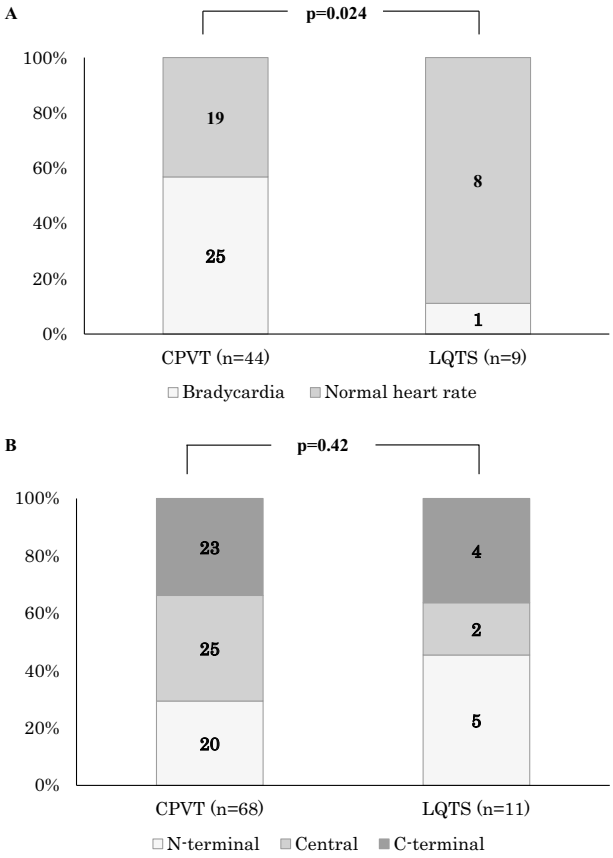

Figure 2. Distribution of bradycardia and *RYR2* mutation locations in patients with CPVT and LQTS. A) Distribution of the bradycardia. The proportion of patients with CPVT having bradycardia was significantly higher than that of patients with LQTS. B) Mutation locations in RYR2. There were no significant differences in the locations of *RYR2* mutations between CPVT and LQTS.

tients with LQTS (n=11) had a significantly longer QTc interval and higher Schwartz score than those with CPVT. The median QTc interval in patients with LQTS was 480.0 (460.0-515.0), and the mean Schwartz score was 4.7±1.5. In contrast, in CPVT patients, the median QTc interval was 411.5 (392.8-433.3), and the mean Schwartz score was 2.3±0.9.

Among the 79 probands, 29 CPVT patients (43%) and 3 LQTS patients (27%) experienced fatal cardiac events that required cardiopulmonary resuscitation (CPR), showing no significant differences in the disease severity between the 2 phenotypes (p=0.51).

Heart rate in RYR2 mutation carriers

We excluded 26 patients (CPVT, n=24; LQTS, n=2) from the analysis of the heart rate because we could not obtain their ECGs before the administration of β-blockers. Fig. 1 shows the relationship between the heart rate at rest and the age of ECG recording in patients with CPVT and LQTS. The heart rates in CPVT patients (open circles) were lower than those of LQTS patients (filled circles), especially at older ages. Fig. 2A depicts the frequency distribution of patients showing bradycardia: 25 out of 44 CPVT patients (57%) vs. 1 out of 9 LQTS patients (11%). Thus, the proportion of patients with CPVT having bradycardia was significantly higher than that of patients with LQTS (p=0.024).

Location of mutations

Fig. 2B shows the frequency of the three different locations of *RYR2* mutations described above. Regarding how the distribution of *RYR2* mutation sites differed between the

two different phenotypes, no significant differences were found in the mutation sites.

Discussion

For several years, *RYR2* mutations have been regarded as specific for CPVT. However, NGS has enabled us to identify *RYR2* mutations in other phenotypes as well, such as LQTS. Therefore, we can no longer diagnose a patient with CPVT simply because they carry an *RYR2* mutation. In this study, we analyzed the phenotypical and genotypical characteristics of 79 probands with *RYR2* variants whose diagnoses were either CPVT or LQTS. To differentiate CPVT from LQTS, we used modified Schwartz scores (11). Regarding the Schwartz score definitions, patients with a score ≥3.5 were deemed likely to have LQTS. However, we detected 8 CPVT patients with a score ≥3.5 and with bVT at ECG. Our data suggest that some CPVT patients with higher Schwartz scores might be misdiagnosed as LQTS despite showing bVT, which is specific for CPVT.

A previous (12) stated that one characteristic finding of CPVT on resting ECG is bradycardia, which may aid in the diagnosis of CPVT. In the present study, we found that bradycardia was more frequent in patients with CPVT than in those with LQTS. We also hypothesized that the locations of *RYR2* mutations might be useful for distinguishing CPVT from LQTS, but the mutation locations were not associated with the phenotype in this study. Taken together, our findings suggested that the detection of *RYR2* mutations during the genetic analysis may indicate the presence of CPVT, even in patients with a modified Schwartz score ≥3.5, particularly when the patient exhibits bVT or pVT and bradycardia on ECG.

There are no international diagnostic criteria for distinguishing CPVT from LQTS, and it is difficult to conclusively diagnose patients who have both QT prolongation and bVT or pVT with either of these diseases. There may even be a combined arrhythmic disease with a phenotype of both CPVT and LQTS. In the present study, however, we differentiated our patients' diagnoses based on the definition shown in the Methods section. Further studies will be needed to establish diagnostic criteria for combined arrhythmic diseases.

Immediately after recovery from a critical cardiac event, the QTc intervals are often prolonged. Therefore, we cannot decide whether a patient has CPVT or LQTS based solely on the QTc interval immediately after a cardiac event,. Although the exercise stress test (EST) may offer another reliable tool for a differential diagnosis, exercise itself also significantly prolongs the QTc intervals in *RYR2*-positive cases, as reported by Medeiros-Domingo et al. (15). In addition, the EST is not suitable for certain patients, such as those with severe brain injury. For these reasons, CPVT patients may be misdiagnosed with LQTS (2).

Study limitations

In this study, we included all of the *RYR2* variants with MAFs <0.005, even if the variants were predicted to be benign by an *in silico* evaluation. Thus, some of the variants might not have affected the phenotype of the patients in this study. In addition, our population was relatively small, and further studies in larger populations will be needed to confirm the phenotypical differences between CPVT and LQTS patients with *RYR2* mutations.

Conclusion

Among 79 *RYR2* mutation carriers, bradycardia was more frequent in patients with CPVT than in those with LQTS. This difference may be useful for distinguishing CPVT from LQTS induced by *RYR2* mutations. With the advent of NGS, we should be careful when diagnosing carriers of *RYR2* mutations, especially those who show bradycardia.

The authors state that they have no Conflict of Interest (COI).

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