A Common Mutation of Long QT Syndrome Type 1 in Japan

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Background: Previous studies of long QT syndrome (LQTS) have revealed the presence of country-specific hot spots in KCNQ1 mutations, and the purpose of this study was to evaluate the influence of a common mutation on clinical phenotypes in Japanese LQT1 patients.

Methods and Results: We retrospectively studied the frequency of each mutation in 190 LQT1 Japanese probands and evaluated the clinical severity of LQT1 among carriers with a common mutation. We also compared it with that of carriers with other mutations. In the Japanese cohort, the most common mutation was p. A344spl (c.1032 G>A), comprising a substitution of a guanine for an adenine at the last base of exon 7, and it was found in 17 probands (8.9%). Regarding the clinical characteristics of A344spl carriers, the mean age-of-onset was 10±4 years, >40% were symptomatic, and the mean corrected QT interval was 46±30 ms. The prognosis for carriers of the A344spl mutation (n=31) was intermediate between that for the A341V mutation reported to be associated with severe phenotypes (n=24) and other mutations (n=290).

Conclusions: The A344spl mutation was a frequent LQTS genotype in Japan, which indicates that the influence of country-specific hot spots should be considered when studying LQT1 clinical phenotypes. (Circ J 2015; 79: 2026–2030)

Key Words: KCNQ1; Long QT syndrome; Mutations

The inherited arrhythmogenic disorder long QT syndrome (LQTS) is characterized by prolongation of the QT interval, syncope, torsade de pointes, and sudden cardiac death (SCD).1-3 There have been 16 candidate genes identified4 and KCNQ1, which encodes the α subunit of the slow delayed rectifier potassium channel (Kv7.1), is the most commonly mutated gene, causing LQTS type 1 (LQT1).5 Several reports have described common mutations in LQT1. A mutation located in the S6 segment in the KCNQ1 channel, A341V, was identified in a South African population,6,7 and was linked to a severe phenotype with a high incidence of sudden death. In contrast, Y111C in Sweden8 and G589D in Finland9 are relatively benign mutations associated with a low incidence of cardiac arrhythmia events in heterozygous individuals. These findings suggest that hot-spot mutations differ between countries, so we studied a common mutation in a Japanese LQT1 cohort and evaluated its influence on clinical phenotypes in Japanese LQT1 patients. The primary objective of this study was to evaluate the clinical severity of LQT1 among A344spl genotype-positive patients, and to compare it with that of A341V genotype-positive patients and other mutation carriers, in a Japanese population.

Methods

Study Population
Shiga University of Medical Science (Otsu), Kyoto University (Kyoto) and the National Cerebral and Cardiovascular Center

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Common LQT1 Mutation in Japan

Molecular Genetics Analysis

Mutation analysis was conducted as previously described.\textsuperscript{13,14} The study received institutional review board approval and written informed consent was given by each patient or their guardians, according to standards approved by local institutional review boards. Briefly, genomic DNA was isolated from peripheral blood leukocytes. Screening for LQTS-causing genes (\textit{KCNQ1}, \textit{KCNH2}, \textit{SCN5A}, \textit{KCNE1}, \textit{KCNE2} and \textit{KCNJ2})\textsuperscript{15-18}

(Suita) are based in regional referral centers that offer comprehensive clinical and genetic testing, enabling highly specialized treatment of inherited arrhythmias. The model of these clinics facilitates the detection of genetic patterns in Japan. Probands (n=190) were defined as the first mutation-carrier identified in an index family. Patients with either double mutations in \textit{KCNQ1} or accompanying mutations in other genes were excluded from the clinical analysis.\textsuperscript{11,12}

\textit{Figure 1.} LQT1 mutations in each country. A summary of \textit{KCNQ1} mutations found in more than 2 probands in Japan, France and Germany (current study), and those in more than 5 probands in the United States and the Netherlands (Kapplinger et al.\textsuperscript{25}). LQT1, long QT syndrome type 1.
was performed using polymerase chain reaction/single-strand conformation polymorphism or denatured high-performance liquid chromatography analyses and then DNA sequencing in each center.

Clinical Phenotypes in LQT1
The 345 patients in the Japanese cohort were classified as either symptomatic or asymptomatic, based on prior experience of cardiac events (syncope, cardiac arrest (CA), SCD). SCD in a first-degree relative under 40 years old was deemed to be LQTS-related, even in the absence of direct genotyping and/or ECG documentation. Markers of clinical severity were considered to be the proportion of symptomatic mutation carriers, the incidence of life-threatening arrhythmias, age at first cardiac event, and cardiac events before commencing β-blocker therapy, through to 40 years of age. Schwartz scores were also calculated. 19 ECG parameters were measured at baseline, and included RR, QT (QRS onset to the end of the T-wave interval), QTpeak (QRS onset to the peak of T wave), and QTpeak-end (the peak of T wave to the end of T wave) intervals, as previously described. 12 The QT interval was corrected by Bazett's formula. 20

Statistical Analysis
Categorical variables are presented as absolute and relative frequencies, and compared using the chi-squared test without Yates continuity correction. Gene variation between each center was compared with Fisher's exact test. Clinical characteristics of genotype groups were compared using Student's t-test as appropriate for continuous variables, which are expressed as the mean and standard deviation. Event-free survival rate was described by Kaplan-Meier cumulative estimates, with comparisons performed by the log-rank test. Time from birth to first event up to 40 years of age was considered for any events, and for CA/SCD. A 2-tailed value of P <0.05 was considered to denote a statistically significant difference. Statistical analysis was performed with SPSS Statistics Version 22 (IBM Corporation, Somers, NY, USA).

Results
Most Common Mutation in the Japanese LQT1 Cohort
Figure 1 shows the distribution of LQT1-related mutations identified at the 3 genetic centers in Japan. Among these, the most frequent was p. A344splt, a splicing mutation involving the substitution of the last base of exon 7 from guanine to adenine (c. 1032 G>A). The A344splt mutation was present in 17 probands (8.9%) in a heterozygous condition.

Typical LQTS Phenotype of A344splt Carriers
In 31 carriers from 17 families with A344splt, the mean age-of-onset was 10±4 years old. A history of cardiac symptoms was present in 13 patients (42%), of whom 11 (36%) had experienced symptoms before 15 years of age; 3 of the 31 carriers (10%) had CA or SCD. Exercise was the major trigger of lethal arrhythmias, being the cause of cardiac events in 11 of the 13 symptomatic patients (85%). The mean corrected QT (QTc) interval in patients with A344splt was 461±30 ms. Using the Schwartz criteria, 17 of 31 patients (55%) had at least 3.5 points, a score that is definitive of LQTS. 19

Table shows the clinical characteristics of 344 Japanese patients harboring the A344splt mutation, which causes a splicing error at exon 7-intron 7, KCNQ1

<table>
<thead>
<tr>
<th>Table. Clinical Characteristics of Carriers of A344splt, A341V and “Other” Mutations of Long QT Syndrome</th>
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<tbody>
<tr>
<td>Genotype-positive, n</td>
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<tr>
<td>Female sex, n (%)</td>
</tr>
<tr>
<td>Median age-of-onset, years</td>
</tr>
<tr>
<td>Symptomatic, n (%)</td>
</tr>
<tr>
<td>≤15 years of age, n (%)</td>
</tr>
<tr>
<td>&lt;40 years of age, n (%)</td>
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<tr>
<td>Exercise trigger, n (%)</td>
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<tr>
<td>CA/SCD, n (%)</td>
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<tr>
<td>Heart rate (beats/min)</td>
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<tr>
<td>QTc, ms</td>
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<tr>
<td>Corrected QTpeak, ms</td>
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<td>Corrected QTpeak-end, ms</td>
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<tr>
<td>Schwartz score</td>
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<td>≥3.5</td>
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*% of symptomatic carriers. CA, cardiac arrest; QTc, corrected QT interval; SCD, sudden cardiac death.

Discussion
This multinational study demonstrated that the A344splt KCNQ1 mutation, which causes a splicing error at exon 7-intron 7, 13,21
was common in Japan and the A344spl KCNQ1 mutation was associated with typical LQTS phenotypes.

KCNQ1 is 1 of 3 major genes responsible for LQTS, and it was revealed by the positional cloning method in 1996.\(^6\) KCNQ1 encodes the α subunit of the voltage-gated cardiac potassium channel that underlies the slow delayed rectifier potassium current (I\(\text{Ks}\)). The clinical severity of LQT1 is known to depend on the type and/or location of KCNQ1 mutations. Moss et al demonstrated that mutations of the transmembrane, missense or dominant-negative type were associated with more severe phenotypes than those of C-terminal, non-missense or haploinsufficiency types.\(^7\) KCNQ1 mutations in the cytoplasmic loop were reportedly the most severe because modification of the I\(\text{Ks}\) current by adrenergic stimulation may be impaired.\(^8\)

Therefore, variability in KCNQ1 mutations according to ethnicity or country may affect the outcome of studying clinical phenotypes.

As demonstrated in previous reports, A341V in South Africa,\(^6,7\) Y111C in Sweden\(^9\) and G589D in Finland\(^10\) have been identified as founder mutations with historical data on demographic and clinical characteristics. The A341V mutation is a missense mutation found in South African populations with LQT1, and it is associated with a young age-of-onset, a high frequency of cardiac events, and a longer QTc interval than in non-A341V cohorts. Functional assays also indicated that the A341V phenotype exhibits the dominant-negative effect, with impaired responses to adrenergic stimulation. The Y111C mutation in the N-terminus is frequent in Sweden, and the dominant-negative effect leads to insufficient trafficking of the normal KCNQ1 protein; however, despite marked QT prolongation, the incidence of cardiac events is lower than for A341V (symptomatic carriers, 30% in Y111C vs. 75% in A341V).\(^9\)

In contrast, in Finland, the founder mutation G589D causes a benign phenotype and occurs in 30% of LQTS cases. G589D carriers have mild prolongation of the QT interval (460 ms) under heterozygous conditions, and 26% of patients are symptomatic. These clinical findings are in accordance with an experimental study that confirmed nondominant-negative effects.\(^10\)

The A344spl mutation (c.1032 G>A) was found in all populations, irrespective of country,\(^24\) and may be considered a recurrent mutation. In contrast, the A344spl mutation was significantly more frequent in Japan (8.9%) than in other countries (Figure 1: 5 of 160 LQT1, 3.1% in France; 0 of 81 LQT1, 0% in Germany; and 10 of 465 LQT1, 2.1% in the United States;\(^25\) P<0.05, P<0.01 and P<0.001, respectively). Nevertheless, because it was the most frequent mutation in several Japanese genetic centers, it is likely that related cases exist, as for founder mutations.\(^26\) In vitro and in vivo studies demonstrate that A344spl displays various splicing errors, and leads to the dominant-negative pattern.\(^13\) The clinical severity of LQT1 in A344spl carriers was intermediate relative to carriers of A341V and ‘other’ mutations, which is similar to data from 12 French A344 carriers (clinical characteristics: symptomatic carriers, 42%; mean age-of-onset, 9±5 years; mean QTc, 489±39 ms). The findings of the present study demonstrate that the distribution of common KCNQ1 mutations differs considerably between countries, and when evaluating clinical data, LQT1 phenotypes associated with hot-spot mutations should be identified.

### Conclusions

The A344spl KCNQ1 mutation was significantly more frequent in Japan than in European countries and the United States. Previous reports have shown the presence of country-specific hot spots in KCNQ1 mutations, such as A341V in a South African founder population, Y111C in Sweden and G589D in Finland. A344spl carriers demonstrate typical LQTS phenotypes although the clinical severity was intermediate between A341V, associated with malignant phenotypes, and other LQT1 mutations. Thus the severity of KCNQ1 mutations has been reported to depend on mutation site or type and we should take account of the country when we study the clinical phenotypes of LQT1 corresponding to KCNQ1 mutations.
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Disclosures

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References