L-Type Calcium Channel Mutations in Japanese Patients With Inherited Arrhythmias

Megumi Fukuyama, MD; Seiko Ohno, MD, PhD; Qi Wang, BSc; Hiromi Kimura, MD, PhD; Takeru Makiyama, MD, PhD; Hideki Itoh, MD, PhD; Makoto Ito, MD, PhD; Minoru Horie, MD, PhD

Background: Mutations in genes encoding the L-type cardiac calcium channel (LTCC) are associated with various types of inherited arrhythmias, including Brugada syndrome (BrS). However, the frequency in Asian populations remains unknown. This study aimed to elucidate disease-causing mutations in LTCC-related genes in Japanese patients diagnosed as BrS or idiopathic ventricular fibrillation (IVF), early repolarization syndrome, short QT syndrome, and compare them with those carrying SCN5A mutations.

Methods and Results: We screened CACNA1C and CACNB2b in 312 probands and compared the clinical characteristics between probands with gene mutations in CACNA1C or SCN5A. In results, we identified 6 CACNA1C mutations in 7 unrelated probands and SCN5A mutations in 20 probands. There were no CACNB2b mutation carriers. In topology, half of the mutations were located in the C-terminus. Among 7 CACNA1C mutation carriers, 2 were female and 3 were symptomatic; 2 patients were resuscitated from ventricular fibrillation, and 1 patient had syncope. Compared with SCN5A mutation carriers, there were no significant differences in the ECG characteristics. 2 of 3 symptomatic CACNA1C patients were female, but all female SCN5A mutation carriers remained asymptomatic.

Conclusions: We identified 6 CACNA1C mutations in BrS and IVF patients and their phenotypes were varied. Although mutation frequency was not high, screening of LTCC channel genes may be clinically important to prevent unexpected sudden death. (Circ J 2013; 77: 1799–1806)

Key Words: Arrhythmia; Brugada syndrome; Calcium channel; Genetics; Idiopathic ventricular fibrillation (IVF)

T he cardiac L-type calcium channel (LTCC) plays a pivotal role in the regulation of the plateau phase of the action potential and thereby, the heart rhythm and contractility.1 The LTCC has 4 subunits: α (Cav.1.2) and 3 auxiliary subunits, β2, αδ, and γ. Cav.1.2 constitutes the ion permeating subunit and determines the main biophysical and pharmacologic properties of the channel. CACNA1C encodes Cav.1.2, and CACNB2b and CACNA2D2 encode the β2 and αδ subunits, respectively. The fourth γ subunit is not expressed in the heart.2,3

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In 2004, a CACNA1C mutation (G406R) was identified in multiple patients with a specific subtype of long QT syndrome: Timothy syndrome (LQT8).4 Subsequently, mutations in CACNA1C, CACNB2b and CACNA2D1 have been shown in the patients with Brugada syndrome (BrS), idiopathic ventricular fibrillation (IVF), early repolarization syndrome (ERS),5 and short QT syndrome (SQTS).6–11 Most of the reports are, however, from Western countries and only a few from Asian countries. Therefore, the frequency of LTCC mutations in inherited arrhythmias remains unknown in the Asian population.

The molecular structure of pore-forming α-subunits is similar between calcium and sodium channels (Cav.1.2 and Nav.1.5). In the heart, SCN5A encodes Nav.1.5, and its dysfunction has been shown to be associated with several inherited arrhythmias such as BrS, IVF or ERS.12

The present study aimed to identify disease-causing LTCC mutations in 312 unrelated probands who were diagnosed as BrS, IVF, ERS, or SQTS, and to compare their clinical and ECG characteristics with those of SCN5A mutation carriers. We included patients with SQTS because an earlier report10 on LTCC mutations in patients with BrS reported a significant association with short QT intervals.

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Department of Cardiovascular and Respiratory Medicine, Shiga University of Medical Science, Otsu (M.F., S.O., Q.W., H.K., H.I., M.I., M.H.); Department of Cardiovascular Medicine, Kyoto University Graduate School of Medicine, Kyoto (S.O., T.M.), Japan
Mailing address: Minoru Horie, MD, PhD, Department of Cardiovascular and Respiratory Medicine, Shiga University of Medical Science, Seta-Tsukinowa, Otsu 520-2192, Japan. E-mail: horie@belle.shiga-med.ac.jp
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Methods

Patient Cohort
The study cohort consisted of 312 probands registered in 2 Japanese institutes, the Shiga University of Medical Science and the Kyoto University Graduate School of Medicine, between 1996 and 2012 for the genetic analysis. They were diagnosed as BrS, IVF, ERS or SQTS according to the following diagnostic criteria:13-16 12-lead ECG, and personal/family history of syncope, seizures, or arrhythmic events. Briefly, the ECG criteria for BrS included coved- or saddleback-type ST-segment elevation in at least 1 right precordial lead under baseline conditions, or after pilsicainide challenge test. IVF patients were included to the study if they had experienced one or more VF episodes without any ECG signs of BrS or ERS. Cases of other obvious causes of VF (eg, acute myocardial infarction) were excluded. ERS was diagnosed when the J-point (QRS-ST junction) elevation was ≥0.1 mV in at least 2 inferoposterior leads, manifesting as QRS slurring or notching or a distinct J wave. SQTS was defined as having a corrected QT (QTc) interval ≤360 ms for males, and ≤370 ms for females.

Table 1. Clinical and ECG Characteristics of the Patient Cohort

<table>
<thead>
<tr>
<th></th>
<th>BrS</th>
<th>IVF</th>
<th>SQTS</th>
<th>BrS+SQTS</th>
<th>ERS</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (%)</td>
<td>213 (68.2)</td>
<td>39 (12.5)</td>
<td>10 (3.2)</td>
<td>23 (7.4)</td>
<td>27 (8.7)</td>
<td>312</td>
</tr>
<tr>
<td>Male (%)</td>
<td>191 (89.7)</td>
<td>29 (74.4)</td>
<td>9 (90.0)</td>
<td>20 (87.0)</td>
<td>21 (77.8)</td>
<td>270 (86.5)</td>
</tr>
<tr>
<td>VT or VF (%)</td>
<td>39 (18.3)</td>
<td>39 (100.0)</td>
<td>5 (50.0)</td>
<td>5 (32.7)</td>
<td>14 (51.9)</td>
<td>102 (32.7)</td>
</tr>
<tr>
<td>Age at diagnosis (years)</td>
<td>44.7±16.1</td>
<td>43.1±21.1</td>
<td>31.6±19.7</td>
<td>41.8±12.0</td>
<td>42.9±18.0</td>
<td>43.7±16.9</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>68.6±13.4</td>
<td>72.4±18.3</td>
<td>58.4±10.0</td>
<td>55.3±9.8</td>
<td>65.4±15.1</td>
<td>67.3±14.5</td>
</tr>
<tr>
<td>QT interval (ms)</td>
<td>393.4±63.5</td>
<td>402.8±36.1</td>
<td>316.5±117.3</td>
<td>363.1±36.7</td>
<td>408.3±41.6</td>
<td>387.9±42.0</td>
</tr>
<tr>
<td>QTc interval (ms)</td>
<td>410.9±36.4</td>
<td>433.3±51.5</td>
<td>337.1±30.6</td>
<td>346.8±16.4</td>
<td>416.6±46.6</td>
<td>405.8±44.1</td>
</tr>
</tbody>
</table>

Data are mean ± SD. *P<0.05 vs. BrS, IVF, ERS group. BrS, Brugada syndrome; ERS, early repolarization syndrome; HR, heart rate; (I)VF, (idiopathic) ventricular fibrillation; QTc, corrected QT; SQTS, short QT syndrome; VT, ventricular tachycardia.

Genome Scanning
Genomic DNA was extracted from peripheral blood leukocytes. In addition to the LTCC-related genes (CACNA1C, CACNB2b), we screened SCN5A, KCNQ1, KCNH2, KCNE1-3, KCNE5,17 SCN3B18 and KCNJ19-21 using a high-resolution melting method (HRM)22 or denaturing high-performance liquid chromatography (dHPLC; WAVE system Model 3500, Transgenic, Omaha, NE, USA) and subsequent direct sequencing. Briefly, the coding exons of genes were amplified using primers as previously reported.17,22,24 HRM analyses were performed using the LightCycler®480 (Roche Applied Science, USA). Melting curves were generated by ramping between 64°C and 98°C at 0.02°C/s. Melting curves were normalized between 2 temperature ranges: the leading range and the trailing range.
**CACNA1C Mutations in Japanese Patients**

Fluorescence data were visualized using normalization plotting, and then analyzed using the automated grouping functionality provided by the LightCycler®480 scanning software. The sequences of polymerase chain reaction products showing divergent dHPLC or HRM profiles were directly confirmed by ABI PRISM-3130 sequencer (Applied Biosystems, Foster City, CA, USA). Electropherograms were visually examined for heterozygous peaks and compared with reference sequences for homozygous variations. GenBank accession numbers of CACNA1C were NM_000719 and NM_001167625.1, and NM_201590 for CACNB2b.

**Results**

**Clinical Characteristics**

Table 1 summarizes the clinical characteristics of 312 pro-

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### Table 2. Summary of CACNA1C Mutations and Mutation Carriers

<table>
<thead>
<tr>
<th>Case no.</th>
<th>ECG</th>
<th>Symptom</th>
<th>Family history</th>
<th>HR (beats/min)</th>
<th>PR interval (ms)</th>
<th>QRS interval (ms)</th>
<th>QTc interval (ms)</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BrS</td>
<td>(–)</td>
<td>(–)</td>
<td>73</td>
<td>120</td>
<td>96</td>
<td>392.7</td>
<td>N547S</td>
</tr>
<tr>
<td>2</td>
<td>BrS</td>
<td>VF</td>
<td>(–)</td>
<td>76</td>
<td>200</td>
<td>100</td>
<td>382.7</td>
<td>R632R</td>
</tr>
<tr>
<td>3</td>
<td>54/F</td>
<td>VF</td>
<td>(+)</td>
<td>53</td>
<td>220</td>
<td>134</td>
<td>432.3</td>
<td>R858H</td>
</tr>
<tr>
<td>4</td>
<td>Br/S</td>
<td>VF</td>
<td>(–)</td>
<td>62</td>
<td>200</td>
<td>100</td>
<td>406.6</td>
<td>R1780H</td>
</tr>
<tr>
<td>5</td>
<td>Br/S</td>
<td>(–)</td>
<td>(–)</td>
<td>55</td>
<td>220</td>
<td>80</td>
<td>478.7*</td>
<td>C1855Y</td>
</tr>
<tr>
<td>6</td>
<td>Br/S</td>
<td>Syncope</td>
<td>(+)</td>
<td>67</td>
<td>170</td>
<td>90</td>
<td>433.8</td>
<td>R1910Q</td>
</tr>
</tbody>
</table>

*Excluded from the average. Data are mean ± SD. Abbreviations as in Table 1.

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![Normalized high-resolution melting curves of mutation carriers. Result of the HRM method: (A) case 1, (N547S) mutation in exon 12; (B) case 2, (R632R) in exon14; (C) case 3, (R858H) in exon 19; (D) case 6 (R1910Q) in exon 48. All curves of CACNA1C mutations are significantly different from the wild-type (WT).](image-url)
bands (270 males, 86.5%). Their mean age at diagnosis was 43.7±16.9 years. There were 213 cases of BrS (68.2%), 39 IVF (12.5%), 10 SQTS (3.2%), 23 BrS+SQTS (7.4%) and 27 ERS (8.7%). In each disease category, males were predominant. Mean QT and QTc intervals were significantly shorter and mean heart rates were significantly lower in the SQTS and BrS+SQTS groups than in the others. There were no significant differences for any other measurement.

Identification of Mutations

We identified 6 CACNA1C mutations in 7 probands (2.2%): N547S, R632R (supposed splice error), R858H, R1780H, C1855Y and R1910Q (in 2 probands). There were no CACNB2b mutations found in our cohort. C1855Y carrier was found to have a heterozygous KCNQ1-Y315C mutation and others had no compound mutation in the 8 genes we examined. Because we identified 2 CACNA1C-C1855Y and 1 CACNA1C-R1910Q allele from 632 control alleles, these variants were considered as rare single nucleotide polymorphisms (SNP). Figure 1 shows the topology of Cav1.2 and the location of the variants we found: 3 (50%) were located in the C-terminus, and the remaining 3 in the extracellular, transmembrane and intracellular regions, respectively. Table 2 summarizes the clinical data of these 7 probands: 6 had BrS without QT shortening, and 1 had IVF. Their mean age at diagnosis was 35.6±10.9 years. Mean heart rate was 64.7±9.3 beats/min. Mean QT and QTc intervals were within normal ranges, except for case 5 in which there were compound heterozygous mutations of CACNA1C and KCNQ1. Three probands with R632R, R858H, and R1910Q were symptomatic: 2 were resuscitated from documented VF, and 1 had syncope. The 2 symptomatic patients had a family history. There were 2 female patients (28.6%: IVF and BrS), and both were symptomatic.

Clinical and Genetic Characteristics of 7 Probands

(Figures 2–5)

There were 7 probands in whom we could identify CACNA1C variants displayed varying phenotypes.

Case 1

Heterozygous N547S (c.1540 a>g) was identified in a 29-year-old man (HRM in Figure 2A and sequence in Figure 3A). He had been asymptomatic until this age but his 12-lead ECG showed a coved-type BrS pattern in the precordial leads (Figure 4A). His mutation was found to be inherited from his mother (Figure 5A).

Case 2

Heterozygous R632R (c. 1896 g>a) was identified in a 27-year-old man, (HRM in Figure 2B and sequence in Figure 3B), who was resuscitated from VF and showed a type 2 BrS pattern on ECG (Figure 4B). This variant produces no amino acid change (R632R), but the codon 1896 is the first nucleotide of exon 14 and may cause a splicing error. In LQTS type 1, a KCNQ1-A344A mutation causes LQTS by a similar mechanism.25,26 His mutation was inherited by his daughter (Figure 5B); her ECG was similar to the proband’s ECG, but did not fulfill the diagnostic criteria of BrS.
Case 3 The index proband was a 54-year-old woman who was successfully resuscitated from electrical storm at midnight. Her father had died suddenly at age 46, also at midnight, but no further information was available. Her resting ECG displayed no precordial ST elevation and complete right bundle branch block (Figure 4C). Heterozygous CACNA1C mutation-R858H (c.2339 g>a) was identified in the proband and (Figures 2C,3C) both daughters (Figure 5C).

Case 4 Heterozygous CACNA1C R1780H (c.5339 g>a) mutation was found in a 57-year-old man (Figures 2D,3D). Though asymptomatic, he was diagnosed with BrS (saddleback type) pattern ECG at an annual health check-up. The level of ST elevation in the right precordial leads varied with time (Figure 4D Left), and ST elevation was attenuated by oral cilostazol (100 mg) or intravenous atropine (250 μg) (Figure 4D Right). Unfortunately, we could not get information about his family (Figure 5D).

Case 5 The 5th mutation was CACNA1C-C1855Y (c.5877 g>a), identified in a 26-year-old man with overlapping BrS and LQTS (Figure 3E). He was first diagnosed with LQTS at the age of 6, but his ECG changed to a BrS pattern with age (Figure 4E). He was also found to carry a heterozygous KCNQ1 mutation (p.Y315C), both of which were inherited from his mother (Figure 5C), but her ECG showed only QT prolongation, not BrS pattern.

Case 6 One of the heterozygous R1910Q (c.5729 g>a) carriers was a 30-year-old woman who suffered from recurrent syncope (Figures 2D,3F). Her resting 12-lead ECG was normal, but the sodium-channel blocker, pilsicainide (40 mg IV), revealed a coved-type ST elevation in the right precordial leads (Figure 4F). Her father had died suddenly at the age of 50, but no further information was available (Figure 5F).

Case 7 Another heterozygous R1910Q carrier was a 24-year-old man who remained asymptomatic without a family history of sudden death. His ECG displayed a saddleback-type ST elevation in the right precordial leads. Pilsicainide (50 mg IV) produced a coved-type ST change (Figure 4G). We could not get information about his family (Figure 5G).

Phenotypical Comparison of CACNA1C and SCN5A Variant Carriers
Among the 312 probands with various phenotypes, 7 were found to carry CACNA1C variants, and 6 of them displayed the BrS phenotype with or without symptoms. In contrast, in the same cohort, we identified 20 SCN5A variant carriers. Table 3 compares the clinical features of these carriers with
or IVF patients, not in ERS or SQTS cases. As there were 213 probands with BrS phenotype, the prevalence rates of \textit{CACNA1C} and \textit{SCN5A} variants were 2.8\% and 8.0\%, respectively. Frequencies of carriers displaying the BrS phenotype were approximately the same (\textbf{Table 3}). Regarding ECG characteristics, the type 1 BrS pattern was most seen in \textit{SCN5A} mutation carriers, and type 2 was most frequent in \textit{CACNA1C} mutation carriers. In contrast to a previous report,\textsuperscript{7} QTc intervals in \textit{CACNA1C} variant carriers were not significantly shorter than those in \textit{SCN5A} carriers. Regarding other parameters, there were no significant differences; however, female carriers appeared to be more frequent among \textit{CACNA1C} than \textit{SCN5A} variant carriers (28.5\% vs. 10\%).

\textbf{Discussion}

We identified 6 LTCC-related gene variants in 7 unrelated probands in our cohort containing 5 different phenotypes. LTCC-related gene mutations have been previously found in all the disease categories chosen for this study,\textsuperscript{7,8,15} but we failed to identify LTCC-related variants in our SQTS, BrS+SQTS or ERS cases. Compared with previous reports in Western countries,\textsuperscript{7,8,15} the prevalence of these conditions appeared to be lower in our population.

Our cohort consisted of 270 males and 42 females, and there were significantly more males than females, but the frequency of LTCC variant carriers was higher in females (4.8\%) than in males (1.9\%). In addition, both of the female mutation carriers of \textit{CACNA1C} or \textit{SCN5A} mutation. Of the 20 \textit{SCN5A} variant carriers, 17 showed a BrS phenotype (85\%) and the remaining 3 were IVF patients. Therefore, in our 312 probands, variants in \textit{CACNA1C} and \textit{SCN5A} were all found in either BrS

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
 & \textit{CACNA1C} & \textit{SCN5A} & \textbf{P value} \\
\hline
\textbf{Male} & 5 (71.4) & 18 (90.0) & NS \\
\textbf{BrS} & 6 (85.7) & 17 (85.0) & NS \\
\textbf{Type 1} & 1 & 11 & \\
\textbf{Type 2} & 4 & 3 & \\
\textbf{Type 3} & 1 & 3 & \\
\textbf{IVF} & 1 (14) & 3 (15) & NS \\
\textbf{Symptomatic patients} & 3 (42.9) & 9 (45.0) & NS \\
\textbf{VT/VF} & 2 & 3 & \\
\textbf{Syncope} & 1 & 6 & \\
\textbf{Age (years)} & 35.9±13.3 & 36.5±22.2 & NS \\
\textbf{HR (beats/min)} & 64.7±9.3 & 71.5±15.0 & NS \\
\textbf{PR (ms)} & 178.3±42.1 & 198.1±34.5 & NS \\
\textbf{QRS (ms)} & 95.0±22.0 & 111.4±12.8 & NS \\
\textbf{QTc (ms)} & 406.7±21.8 & 403.2±30.0 & NS \\
\hline
\end{tabular}
\caption{Comparison With \textit{SCN5A} Mutation Carriers}
\end{table}
rriers were symptomatic without showing a BrS pattern ECG. **CACNA1C** R1910Q was identified in 2 probands with different clinical features (Figures 4F-G). 1 showed no remarkable ECG change at rest, but suffered from VP, whereas the other, male R1910Q carrier showed a BrS ECG pattern at rest, but remained asymptomatic. These differences might be caused by the difference in sex. In a previous report on **SCN5A** mutation carriers in BrS, ECG abnormalities in female patients were unclear, compared with male patients. Thus, as far as phenotype is concerned, a BrS ECG pattern also appears to be more prevalent, irrespective of clinical severity, in LTCC variant carriers.

Even among carriers in the same family (Figure 5), phenotypes varied considerably. In families with N547S R632R, and C1855Y (Figures 5A-B-E), the difference appeared to depend on sex. Male carriers were more prone to be symptomatic. In the family with R858H (Figure 5C), 2 daughters of the proband were found to be genotype-positive, but had remained asymptomatic to date, so we need to carefully observe their clinical course.

Regarding the location of **CACNA1C** variants, half of them were in the C-terminus. These C-terminal variants could have some effects on the function of Cav1.2 channels. It has been reported that Cav1.2 affected by mutations in the C-terminus showed dysfunction.

Regarding the **CACNA1C** R632R mutation (Table 2 [case 2]), Figure 1, arginine at codon 632 is located in segment 4 (S4) of domain II (DI). In every domain, S4 is postulated to play a key role as voltage-sensor. Amino acids, arginine (R) or lysine (K) are arranged every third base in S4, forming an α-helix. By sensing the voltage change, S4 transforms to open the gate, thus arginine in S4 is a very important location. Previous functional assays reported that substitution from R or K in S4 to other amino acids in segment 4 significantly reduces the sensitivity to membrane potential.

In the present case 2, the mechanism underlying the dysfunction of Cav1.2 appears somewhat different; substitution of nucleotide guanine to adenine at 1896 did not change the sensitivity to membrane potential. These C-terminal variants could have some effects on the function of Cav1.2 channels. It has been reported that Cav1.2 affected by mutations in the C-terminus showed dysfunction.

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