Genetic Analysis of Brugada Syndrome in Western Japan

— Two Novel Mutations —

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Background  Brugada syndrome is a form of idiopathic ventricular fibrillation characterized by right bundle-branch block pattern and ST elevation in the right precordial leads of the ECG. The SCN5A gene encodes the Î¬-subunit of the human heart sodium channel, which plays a critical role in cardiac excitability, and mutations of SCN5A could underlie Brugada syndrome.

Methods and Results  To detect mutations of SCN5A, DNA samples from 12 Japanese patients with Brugada syndrome were analyzed using direct sequencing. Two patients had novel mutations, G292S and S835L, but no other mutations of SCN5A were detected in the remaining patients. The first mutation, G292S, was identified adjacent to the pore-lining region between the DIS5 and DIS6 transmembrane segments of SCN5A, and the second mutation, S835L, was in the intracellular loop connecting the DIIS4 to DIIS5. Both mutations were not detected in 100 unrelated control subjects.

Conclusion  Two novel SCN5A mutations have been found in Japanese patients with Brugada syndrome.

(Received November 18, 2003; revised manuscript received June 4, 2004; accepted June 8, 2004)

Key Words: Brugada syndrome; Cardiac sodium channel; Polymorphisms; SCN5A gene mutation

Determination of the Brugada-associated mutations in SCN5A in Japanese patients should facilitate pre-symptomatic diagnosis and enable better follow-up of asymptomatic patients. In the present study, we evaluated 12 Japanese patients with Brugada syndrome (9 symptomatic, 3 asymptomatic) for mutations in the translated region of SCN5A.

Methods

Subjects  Twelve, unrelated Japanese patients living in western Japan (11 males, 1 female; age 28–70 years; 9 symptomatic, 3 asymptomatic) diagnosed with Brugada syndrome in the Department of Cardiology, Fukuoka University Hospital, participated in this study. Brugada syndrome was diagnosed by typical ECG findings with syncope and/or documented ventricular tachycardia/fibrillation (VT/VF), and/or inducible ventricular arrhythmias during an electrophysiological study. Patients in whom intravenous Na channel blocker (pilsicainide 1–2 mg/kg body weight, maximum dose, 100 mg) induced typical ECG changes were also included. ECG criteria was based on those reported by Wilde et al: The S wave of V1 and V2 was measured from the tip of r to the tip of r', and the amplitude of the ST segment was measured at the J point. Two cardiologists assessed these measurement without reference to the clinical information of the patients. Ventricular arrhythmia was induced without using any anti-arrhythmic drugs. The criterion for induction of ventricular arrhythmia was sustained polymorphic VT or VF induced by programmed electrical stimulation at the right ventricular apex or outflow. The protocol of ventricular stimulation included up to 3 extra-stimuli being not less than 200 ms and ventricular rate of rapid burst pacing not exceeding 270 beats/min.

In a search for the identical mutation in the general
population, blood specimens of 100 Japanese individuals (70 men, 30 women; age 61±11 years (mean ± SD)) were collected and analyzed. None had Brugada-type ECG findings or a history of VT, VF or syncope.

The study protocol was approved by the Human Ethics Review Committee of Fukuoka University School of Medicine and a signed consent form was obtained from each patient, family members and control subjects.

**Polymerase Chain Reaction (PCR)**

For PCR assay, 16.5 ml (5.5 ml × 3 samples) of peripheral blood was obtained from each subject and immediately stored at –20°C until extraction of genomic DNA using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). We used the primers reported by Wang et al.8 For the PCR amplification of exons 2, 6 and 28 (forward/reverse), exon 20 (forward), and exons 21, 22 and 25 (reverse), the original primers were designed from the intronic sequence (Table 1). All primers were ordered from Hokkaido System Science (Sapporo, Japan). PCR conditions were as follows: an initial denaturation at 95°C for 2 min, denaturation at 96°C for 30 s, annealing at 58–61°C for 30 s, extension at 72°C for 1 min, repeated for 30–35 cycles. After PCR, 10 ml of the reaction mixture was electrophoresed in 5–8% polyacrylamide gel and stained with ethidium bromide.

**DNA Sequencing Analysis**

After removal of excess PCR primers from the reaction mixture with Micro Spin S-300HR Columns (Amersham Biosciences, NJ, USA), direct sequencing was performed with a Big Dye Terminator v3.0 Cycle Sequencing kit and ABI Prism 3100 genetic analyzer (Applied Biosystems, Foster City, CA, USA) according to the manufacturer’s instructions. To confirm the presence of mutation(s), subcloned genomic DNAs in pT7Blue-T-vector (Novagen, Madison, WI, USA) were sequenced. The obtained sequence data were inspected for substitutions from previously reported SCN5A cDNA sequence (GenBank Accession number NM 000335).

**PCR-Mediated Site-Directed Mutagenesis and Restriction Fragment Length Polymorphism**

For the determination of G292S, S835L and H558R, we performed PCR-mediated site-directed mutagenesis. Leukocyte DNA was amplified by PCR using oligonucleotide primers A (5'-CCACCTCTGTTGCGCTACAGTCT-3')8 and B (5'-CAAGCAGTCGGCCTCCACGGCC-3') for G292S, primers C (5'-GAGCCAGAGACCTTCACAGGGTCCCT-3')8 and D (5'-AGTGTCAGGTTCCCCAGTCGGGCCC-3') for S835L, and primers E (5'-AGCGGGAGAGCGAGAGCCT-3') and F (5'-CTGTCCCTGGGCACTGGTCACG-3') for H558R, respectively. The 3' ends of primers B, D and E included the nucleotides CGC, ATC and AGC rather than the native AGC, ACT and ACC bases in order to generate novel restriction sites for BbeI (GGCGCC), MseI (TTAA) and HhaI (GCGC), respectively. The P1090L polymorphism was confirmed by restriction fragment length polymorphism. Leukocyte DNA was amplified by PCR using primers G (5'-AGGGTCTAACCCCCAGGGTCA-3') and H (5'-CCCAGCTGGCTTCAGGGACCA-3')8 Amplifications using primer pairs were conducted by 30 cycles of 96°C for 30 s, 60°C for 30 s, and 72°C for 1 min using a thermocycler. The PCR products digested by restriction enzymes were determined by 8% polyacrylamide gel electrophoresis. DNA was stained with ethidium bromide and visualized by ultraviolet transillumination.

### Table 1  PCR Primers of the SCN5A Gene

<table>
<thead>
<tr>
<th>Exon</th>
<th>Forward primer</th>
<th>Reverse primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>CTTCCCTCACACGACAGC</td>
<td>ATGAGCCACCCTCTAAATGAGCC</td>
</tr>
<tr>
<td>6</td>
<td>CTTCTCGATGTTGCTCTCC</td>
<td>AAGGGATTCTGTTGACACAGC</td>
</tr>
<tr>
<td>20</td>
<td>CGCCACCCCCATCATCTAG</td>
<td>GCTGCTGCTCTTCCTCCTGTA</td>
</tr>
<tr>
<td>21</td>
<td>CTTCTCGCTGTTGGCACCAG</td>
<td>CTTTCTCTCCTGTTGTTAGTTG</td>
</tr>
<tr>
<td>22</td>
<td>CTCTGCGCTCTCTCTACATGTG</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>CTTCTCCTCCTGTTGAGTTG</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>GCTGCTGCTCTTCCTCCTGTA</td>
<td>GGTATACGCTTGGCGCTC</td>
</tr>
</tbody>
</table>

All primers are shown in the 5’ to 3’ direction.

### Table 2  Clinical Profiles of the Patients With Brugada Syndrome

<table>
<thead>
<tr>
<th>Case</th>
<th>Age/ Sex</th>
<th>Family history</th>
<th>Cardiac symptoms</th>
<th>Documented arrhythmias</th>
<th>Induced arrhythmias</th>
<th>HV (ms)</th>
<th>PQ (ms)</th>
<th>S duration* (ms)</th>
<th>QTc (ms)</th>
<th>ST amplitude* (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F/49</td>
<td>–</td>
<td>Syncope</td>
<td>–</td>
<td>VF</td>
<td>65</td>
<td>157</td>
<td>120</td>
<td>427</td>
<td>0.18</td>
</tr>
<tr>
<td>2</td>
<td>M/37</td>
<td>+</td>
<td>Syncope</td>
<td>–</td>
<td>VF</td>
<td>nt</td>
<td>136</td>
<td>80</td>
<td>393</td>
<td>0.16</td>
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<tr>
<td>3</td>
<td>M/34</td>
<td>–</td>
<td>CPA</td>
<td>VF</td>
<td>nt</td>
<td>170</td>
<td>100</td>
<td>402</td>
<td>0.28</td>
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</tr>
<tr>
<td>4</td>
<td>M/47</td>
<td>–</td>
<td>CPA</td>
<td>VF</td>
<td>nt</td>
<td>164</td>
<td>100</td>
<td>413</td>
<td>0.18</td>
<td></td>
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<tr>
<td>5</td>
<td>M/60</td>
<td>–</td>
<td>Syncope</td>
<td>–</td>
<td>–</td>
<td>64</td>
<td>156</td>
<td>80</td>
<td>387</td>
<td>0.36</td>
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<tr>
<td>6</td>
<td>M/70</td>
<td>–</td>
<td>CPA</td>
<td>VF</td>
<td>nt</td>
<td>176</td>
<td>100</td>
<td>368</td>
<td>0.20</td>
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<tr>
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<td>M/58</td>
<td>–</td>
<td>Syncope</td>
<td>–</td>
<td>VF</td>
<td>57</td>
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<td>90</td>
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<td>8</td>
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<td>–</td>
<td>–</td>
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<td>110</td>
<td>433</td>
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<tr>
<td>9</td>
<td>M/65</td>
<td>–</td>
<td>Syncope</td>
<td>–</td>
<td>VF</td>
<td>43</td>
<td>200</td>
<td>120</td>
<td>422</td>
<td>0.10</td>
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<tr>
<td>10</td>
<td>M/52</td>
<td>–</td>
<td>CPA</td>
<td>VF</td>
<td>nt</td>
<td>48</td>
<td>182</td>
<td>100</td>
<td>391</td>
<td>0.26</td>
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<tr>
<td>11</td>
<td>M/66</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>nt</td>
<td>29</td>
<td>195</td>
<td>100</td>
<td>368</td>
<td>0.20</td>
</tr>
<tr>
<td>12</td>
<td>M/28</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>nt</td>
<td>140</td>
<td>100</td>
<td>352</td>
<td>0.14</td>
<td></td>
</tr>
</tbody>
</table>

All patients except Case 6 are still alive. A cardioverter defibrillator was implanted in 9 patients, excepting Cases 6, 8, and 12. CPA, cardiopulmonary arrest; VF, ventricular fibrillation; VT, ventricular tachycardia; nt, not tested.

*The S wave of lead V1 and ST-segment elevation of lead V5 in the standard 12-lead ECG at baseline were measured.
Clinical Evaluation

Table 2 summarizes the patient data. Each patient had the ECG findings of Brugada syndrome consisting of RBBB and ST segment elevation in leads V1–3. The ECG findings of Case 11 are shown in Fig 1. Cases 3, 4, 6 and 10 had experienced cardio-pulmonary arrest (CPA) after documented VF (Table 2). Syncopal episodes had occurred in patients except for case nos 8, 11 and 12 (asymptomatic). Cases 2 and 11 had a family history of sudden death. The S wave duration in lead V1 recorded in all patients was greater than 80 ms. All patients except Cases 2, 9 and 12 showed ST segment elevation of more than 0.18 mV in lead V2 at baseline. Programmed ventricular stimulation induced VF or VT in 8 of the 12 patients (Table 2). All patients except Case 7 showed obvious ST segment elevation following intravenous administration of pilsicainide, and VF was induced by intravenous administration of pilsicainide in Case 1.

A cardioverter defibrillator was implanted in 9 patients, but not Cases 6, 8, and 12. Of these, Cases 6 and 12 were asymptomatic and Case 8 died before the implantation of ICD because of cerebral hemorrhage 3 days after admission to the hospital. Eleven patients showed no cardiac abnormalities on physical examination and echocardiography. Case 11 was a 66-year-old male with acute non-Q myocardial infarction, after which the ECG showed RBBB and saddleback type ST-segment elevation. Intravenous administration of sodium channel blocker (pilsicainide 50 mg) resulted in elevation of the J-wave by more than 0.2 mV. VF was induced by programmed electrical stimulation.

Results
The 12 patients with clinical evidence of Brugada syndrome and 4 controls were sequenced for mutations in the translated regions of \( \text{SCN5A} \) (Table 3). Novel mutations were identified in 2 cases (Cases 1 and 11), and 2 polymorphisms, H558R and P1090L, were identified in 3 other patients (Cases 7, 8 and 9) and 1 control subject (Table 3).

In Case 11, an abnormal conformer was identified in exon 7 of \( \text{SCN5A} \) (Fig 2Upper). DNA sequence analysis revealed a G \( \rightarrow \) A base substitution (G874A) in exon 7 leading to the substitution of glycine with serine at codon 292, which lies in the first P segment adjacent to the pore-lining region between the DIS5 and DIS6 transmembrane segments of the cardiac sodium channel (Fig 3). The P segment is a conserved region within the Na\(^+\)-channel family. PCR products, generated using primers A and B as described in the Methods section and digested with \( \text{BbeI} \), were electrophoresed onto 8% polyacrylamide. Lane M, marker from a digest of pBR322; lane 5, Case 11; remaining lanes, relatives of Case 11. DNAs of lanes 2, 4–8 subjected to \( \text{BbeI} \) digestion showed a 312-bp and 293-bp bands, indicating loss of the \( \text{BbeI} \) site (heterozygote). DNAs of lanes 1 and 3 subjected to \( \text{BbeI} \) digestion showed a 293-bp band resulting from complete digestion of \( \text{BbeI} \) (wild type).

Mutation Analysis

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Fig 3. Schematic representation of the 4 homologous domains (DI–DIV) of the \( \beta \)-subunit of the cardiac Na\(^+\) channel, \( \text{SCN5A} \)-encoded protein. The locations of the G292S and S835L mutations are indicated by arrows.

Fig 4. Detection of mutation in the family members of Case 11. (A) Restriction fragment length polymorphism analysis of the G to A substitution at codon 292. The PCR products, generated using primers A and B as described in the Methods section and digested with \( \text{BbeI} \), were electrophoresed onto 8% polyacrylamide. Lane M, marker from a digest of pBR322; lane 5, Case 11; remaining lanes, relatives of Case 11. DNAs of lanes 2, 4–8 subjected to \( \text{BbeI} \) showed 312-bp and 293-bp bands, indicating loss of the \( \text{BbeI} \) site (heterozygote). DNAs of lanes 1 and 3 subjected to \( \text{BbeI} \) digestion showed a 293-bp band resulting from complete digestion of \( \text{BbeI} \) (wild type). (B) Pedigree of Case 11. (Circles) Females; (Squares) males; SD, sudden death. Numbers underneath symbols represent the age of the individual at the time of analysis. Mutation carriers are marked with solid symbols; non-gene carriers are marked with open symbols. The proband is indicated by an arrow.
costal V1 lead at baseline. Electrocardiographic examination could not be performed in 1 sister (II-4).

In Case 1, we found a serine to leucine substitution at codon 835 of SCN5A caused by a C to T change at nucleotide position 2504 of the SCN5A cDNA (Fig 2Lower). This mutation lies in the intracellular loop between DIIS4 and DIIS5 (Fig 3). Our request to analyse the family members of Case 1 was refused, so to confirm the missense mutation and polymorphisms, site-directed mutagenesis and restriction enzyme analysis were performed. PCR products generated with primers C and D created a MseI site, TTAA with S835L mutant (Fig 5A), and those using primers E and F created a HhaI site, GCGC with H558R polymorphism (Fig 5B), respectively. To confirm P1090L polymorphism, the PCR products using primers G and H were digested by restriction enzyme MspI (Fig 5C).

Analysis of 100 control individuals did not identify the novel mutations (G292S and S835L); the allelic frequencies of the H558R and P1090L polymorphisms were 0.12 and 0.02, respectively. Although some of the SCN5A mutations associated with Brugada syndrome render the Na channel

Discussion

In the present study, the genetic nucleotide sequence of SCN5A was analyzed in both Japanese patients with Brugada syndrome and control subjects. We identified 2 novel single missense mutations, G292S and S835L, which to our knowledge have not been previously reported in unrelated Japanese Brugada patients. Furthermore, 2 polymorphisms, H558R and P1090L, were found in both patients with Brugada syndrome and normal controls. Iwasa et al reported that the allelic frequencies of H558R and P1090L polymorphisms in Japanese population were 0.08 and 0.04, respectively, and we found similar allelic frequencies in our control subjects. Although all 28 exons and adjacent regions of SCN5A in the 12 patients were sequenced, no additional pathological mutations could be detected. Mutations of SCN5A are found in only a minority (15–20%) of patients with Brugada syndrome.

Genetic variants of SCN5A, which encodes the cardiac sodium channel Î±-subunit, are associated with Brugada syndrome and the LQT-3 subtype of long-QT syndrome. Brugada syndrome and LQT-3 comprise more than 60 SCN5A mutations, most resulting in single amino acid substitutions. Although some of the SCN5A mutations associated with Brugada syndrome render the Na channel...
entirely nonfunctional, many of the mutations reduce the Na⁺ current via abnormalities in the inactivation properties of the Na⁺ channels. The reduction in Na⁺ current is thought to suppress the action potential plateau in cells, such as those in the right ventricular epicardium. Preferential suppression of the action potential plateau in epicardial cells will generate a potential gradient that can manifest as ST-segment elevation on the ECG. Unlike Brugada syndrome, SCN5A mutations with LQT-3 result in partial failure of inactivation, causing a small fraction of Na channels to remain continually open, which leads to persistent Na⁺ current during the action potential plateau and results in increased duration of the QT interval on the ECG. In the present study, the QT interval of patients with Brugada syndrome was within the normal range (Table 2).

Asymptomatic subjects with Brugada-type ECG have been found (12 of 8,612 (0.14%) Japanese individuals), although the clinical significance of the Brugada-type ECG findings in asymptomatic subjects is unclear. It has been reported that the QT interval and PQ interval (≥210 ms at baseline, and excessive QRS interval prolongation after Na⁺-channel blockade, was more likely to be found in patients with Brugada syndrome who were carriers of an SCN5A mutation. In our study, however, 2 patients showed prolonged His-ventricular interval and only 1 of them had a SCN5A mutation (S835L), and none of the patients with Brugada syndrome showed prolonged PQ interval (Table 2). Morita et al reported that the incidence of ST level >0.15 mV at baseline, with additional ST elevation following pilsicainide injection (>0.10 mV) in lead V₂, was useful for detecting programmed electrical stimulation-induced VF in asymptomatic subjects with Brugada syndrome. Atarashi et al pointed out that S wave width of ≥0.08 s in V₁ and ST elevation of ≥0.18 mV in V₂ were predictive for VF in patients with Brugada syndrome. In the present study, all patients had a wide S wave (≥0.08 s) in V₁ and 9 of the 12 patients had high ST amplitude (≥0.18 mV) in V₂ (Table 2).

The SCN5A channel consists of 4 homologous domain (DI–DIV) joined by linking intracellular domains (Fig 3), each of which contains 6 transmembrane segments (S1–S6). Several reports have shown that mutations in the pore region, the S6, the S4–S5 loop, and the S5–S6 loop in domain I correlate with reduced current. The first mutation in the intracellular loop of domain I. Priori et al reported that R282H, V294M and G319S mutations with Brugada syndrome were in the S5–S6 loop of domain I, and S835L found in Case 1, who had an episode of VF, but no demonstrable structural heart disease, was the first mutation in the intracellular loop of domain I. Priori et al reported that R282H, V294M and G319S mutations with Brugada syndrome were in the S5–S6 loop of domain I, and 16 of 28 SCN5A mutations with Brugada syndrome were found in the extracellular (n=10) and intracellular (n=6) loops! The I4 subunit of the sodium channel is highly conserved in various species. We searched the SCN5A homologues in 3 different species (mouse, rat, cattle) for sequence changes in the G292 and S835, but no sequence variant was found at codon 835, suggesting that the position is conserved. G292 was consistent with those of the mouse and rat, and the position in cattle was alanine, which is an aliphatic amino acid similar to glycine. In the family of Case 11, an ECG abnormality was observed in a G292S carrier (III-2) and 3 siblings (II-2, II-5 and II-8) were reported to have died suddenly. Thus, the S835L and G292S changes are likely to be mutations and not polymorphisms.

A recent study indicated that the common polymorphism H558R, which is present in 20% of the population, could mitigate the effects of a nearby mutation (T512I) on Na⁺-channel function. However, we could not find any other mutations in the patient with H558R polymorphism.

Genetic heterogeneity of Brugada syndrome has been demonstrated, but other causally related genes await identification. Genetic heterogeneity has been also identified by linkage to a region on chromosome 3 (3p22–25) in a family with Brugada syndrome. Although the functional role of this gene locus has not been defined, the study suggests that other as yet unidentified genes could play a role in the manifestation of disorders of cardiac excitability. We found 2 novel SCN5A mutations and no other pathogenic mutations were found in the remaining 10 patients with Brugada syndrome. Our findings highlight the existence of a variety of pathogenetic genes responsible for Brugada syndrome.

Acknowledgments

The authors thank Mie Morita for her expert technical assistance. This work was partly supported by grants from the Japanese Ministry of Education, Science, Sports and Culture (No. 14571115).

References


