Unique clinical characteristics and $SCN5A$ mutations in patients with Brugada syndrome in Taiwan

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Background/Purpose: Brugada syndrome (BrS) is a heritable sudden cardiac death (SCD). Mutations in the $SCN5A$ gene (the most common BrS-causing gene) are responsible for 20–25% of this disease in Caucasian populations. However, the prevalence of $SCN5A$ mutations in patients with BrS in the Chinese Han population in Taiwan remains unknown. Therefore, in this study, we investigated the prevalence of the $SCN5A$ mutation in the largest BrS cohort in Taiwan.

Methods: We consecutively enrolled 47 unrelated patients with BrS from medical centers and hospitals in Taiwan between 2000 and 2010. Mutations within all the 27 translated exons, and exon–intron boundaries of the $SCN5A$-encoded cardiac sodium channel were screened in all patients with BrS using direct sequencing. A total of 500 unrelated healthy volunteers with a normal electrocardiogram were genotyped as a control group.
Introduction

Brugada syndrome (BrS) is a sudden unexpected cardiac death. In 1992, Brugada first described eight patients with a history of sudden death and a distinct electrocardiogram (ECG) pattern, consisting of right bundle branch block (RBBB) with ST-segment elevation in the V1, V2, and V3 leads and a normal QT interval in the absence of any structural heart disease.\(^1\) BrS is known to occur in 1–5/10,000 inhabitants worldwide. Its frequency is lower in Western countries and higher (≥5/10,000) in Southeast Asia, especially in Thailand and the Philippines, where BrS is considered to be the major cause of sudden death in young individuals. In these countries, the syndrome is often referred to as sudden unexplained nocturnal death syndrome.\(^2\) Results of epidemiologic studies suggest that the disease might be responsible for nearly half of all the sudden cardiac deaths (SCDs) in individuals without structural heart disease and that it is the most common cause of SCD in young adults in South Asia.\(^3\,^4\)

The clinical presentations of BrS are syncope, seizure, and sudden death usually occurring early in the morning. The life-threatening arrhythmia of BrS is usually ventricular fibrillation (VF) or polymorphic ventricular tachycardia (VT).\(^5\) The average age at the time of initial diagnosis or sudden death is 46 ± 7 years, especially in men in our previous report.\(^6\) This syndrome generally does not have any prodromes and the patients usually do not have any cardiovascular diseases.

In 1998, Chen et al first reported that BrS has a genetic basis that is linked only to mutations in the sodium channel, voltage-gated, type V, alpha subunit (SCN5A) gene, which encodes the β-subunit of the sodium channel\(^6\) and has been shown to be responsible for 20–25% of the disease in Caucasian populations.\(^9\) It is a hereditary disease, having an autosomal dominant inheritance pattern with incomplete penetrance. Among the Asia countries, the disease has been reported in Japan, Thailand, India, Laos, Vietnam, Singapore, and Cambodia.\(^10,11\) Studies from Beijing, Nanjing, Hangzhou, and Hong Kong showed that the prevalence of SCN5A mutation in the Chinese Han population varied from 0% to 25%.\(^12–16\) However, the percentage of SCN5A mutations in patients with BrS in the Chinese Han population in Taiwan remains unknown.

Since 2000, we have been continuously collecting information about the patients diagnosed with BrS at the National Taiwan University Hospital (NTUH). In addition, patients with BrS were referred from many medical centers and hospitals in Taiwan for genetic screening for more than 10 years. With this support, we have collected the data on most patients with BrS in Taiwan. In this study, using these data, we report the unique clinical characteristics and SCN5A genetic information of patients with BrS in this largest cohort in Taiwan.

Methods

Study patients

From 2000 to 2010, we consecutively enrolled 47 unrelated patients with BrS from medical centers and hospitals in Taiwan. BrS was definitively diagnosed based on a consensus report in 2005 when a Type 1 ST-segment elevation (Brugada ECG) was observed in more than one right precordial lead (V1–V3) in the presence or absence of a sodium-channel blocking agent and in conjunction with at least one of the following criteria: documented VF, polymorphic VT, a family history of SCD (<45 years old), coved-type ECGs in family members, inducibility of VT with programmed electrical stimulation, syncope, or nocturnal agonal respirations.\(^17–20\) We collected basic clinical information including age, sex, past history (including syncope or sudden cardiac arrest), circumstances surrounding these events (including sleeping, working, resting, or exercise), and any prodromes or family history of SCD (<45 years old). The study was approved by the local ethical committee of NTUH, and all study patients provided informed consent.

Genetic screening for SCN5A mutations

DNA extraction: After blood collection, samples were immediately placed into tubes with ethylenediaminetetraacetic acid reagent and preserved in ice. The whole blood samples were centrifuged at 2000 revolutions per minute (rpm) for 10 minutes under 25°C and three distinguishable fractions were obtained. The buffy coat, which was located in the intermediate layer, was drawn into another 15-mL centrifuge tube for further isolation procedures using Gentra Puregene Blood Kits (Qiagen, Hilden, Germany). First, the red blood cell (RBC) lysis solution was added into
theuffy coat to remove contaminants from the RBCs. Subsequently, the samples were centrifuged at 3000 rpm for 10 minutes and the supernatant liquids were discarded to yield pure buffy coat without RBCs. Next, the cells were mixed with cell lysis solution to induce chemical breakdown. The genomic DNA was obtained in the supernatant after centrifuging at 3000 rpm for 10 minutes. Finally, the genomic DNA pellet was precipitated and washed using isopropanol and ethanol solutions, respectively. After a dry bath for 10 minutes, the DNA pellet was dissolved in 500–800 μL ddH2O.

Polymerase chain reaction and direct sequencing

The DNA obtained from patients with BrS was subjected to polymerase chain reaction (PCR), which was performed in thin-walled PCR tubes having a total volume of 25 L containing 100 ng of genomic DNA, 0.12 μM of each primer, 100 μM deoxyribonucleotide triphosphates, 0.5 U AmpliTaq Gold (PE Applied Biosystems, Foster City, CA, USA), and 2.5 L of GeneAmp 10× buffer II (10 mM Tris-HCl, pH = 8.3, 50 mM KCl) in 2 mM MgCl2 as provided by the manufacturer. Amplification was performed in a multiblock thermal cycler (Thermo Hybaid, Ashford, UK). PCR amplification was performed with an initial denaturation step at 95°C for 10 minutes, followed by 35 cycles involving denaturation at 94°C for 30 seconds, primer annealing at 55°C for 30 seconds, and an elongation step at 72°C for 1 minute unless otherwise specified in the Electronic Supplementary Material (S1). The final extension step was 72°C for 10 minutes. The primers were designed to cover the entire coding region of the genes based on the information in previous studies as well as the sequence data in GenBank. Multiple primer pairs were used for large exons. The amplicons of SCN5A genes were sequenced with dye-terminator cycle sequencing method (Perkin-Elmer, Foster City, CA) on an automatic sequencing apparatus (ABI Prism 373A sequencer, Perkin-Elmer).

Mutations or single nucleotide polymorphisms (SNPs) within all 27 translated SCN5A-encoded cardiac sodium channel NaV1.5 were detected using direct sequencing. Five hundred unrelated patients with BrS who were enrolled, and 14 patients were identified with SCN5A genetic variants. The clinical characteristics of these 14 BrS patients with SCN5A genetic variants (12 men and two women) are summarized in Table 1. The average age at the time of syncope, seizure, or cardiac arrest was 40 ± 13 years (range: 18–70 years). All of them had normal physical examinations and none had significant biochemical abnormalities that could be correlated with electrical events. Cardiac catheterization and echocardiography of these patients did not reveal any coronary artery disease or obvious structural heart disease. Only three patients (21.4%) had a positive family history of SCD. Four patients experienced SCD, and six presented with seizure or syncope. The circumstance of the index events was mostly resting (12/14, 86%) rather than sleeping. Ten patients had a spontaneously Brugada-type I ECG and seven patients received implantable cardioverter-defibrillator implantation.

Genetic variants of the SCN5A gene

Of the 14 BrS patients with SCN5A variants, four had homozygous missense mutations and 10 had heterozygous nonsynonymous SNPs in the SCN5A gene, compared with the genetic variants in the 500 unrelated controls without BrS. The prevalence rate of SCN5A mutations was approximately 8% (4/47, 95% CI = approximately 0.03–0.13). In total, there were three different homozygous missense mutations and two heterozygous nonsynonymous SNPs that resulted in five different amino acid changes (Table 2). The homozygous missense mutations (1651 G>A, 1776 C>G, 3578 G>A) produced three different amino acid changes (Arg551Thr, Asn592Lys, and Arg1193Gln) and were located in exons 12 and 20 of the SCN5A gene individually (Fig. 1). Four patients with BrS had heterozygous functional SNPs in exon 12 (1673 A>G) that caused a fourth amino acid change (His558Arg) and six patients with BrS had heterozygous nonsynonymous SNPs in exon 18 (3269 C>T) that caused the fifth amino acid change (Pro1090Leu) (Fig. 2). Interestingly, three BrS patients with a family history of SCD did not have an SCN5A mutation or nonsynonymous SNPs. In contrast, four BrS patients with SCN5A mutations did not have a family history of SCD. A comparison of the prevalence of BrS patients with SCN5A mutations in Caucasian populations with that in the Chinese Han population in Taiwan showed that the prevalence of BrS patients with SCN5A mutations was significantly lower (8.5% vs. 25%, p = 0.0007) (Fig. 3).
Comparisons of prevalence of SCN5A mutations in patients with BrS from different studies in the Chinese Han population

We listed several studies on patients with BrS in Chinese Han populations in Table 3.12 The patients with BrS enrolled in these studies are all Chinese Han from northern and southern China. The number of enrolled patients varied from 7 to 50 (<15 patients in three studies). Two studies enrolled symptomatic and asymptomatic patients with BrS, whereas three only enrolled symptomatic patients with BrS. The study from Hangzhou showed that five SNPs were detected in 48 patients with BrS, but the prevalence rate of SCN5A mutation was 0%, whereas the study from Hong Kong revealed that the prevalence rate of SCN5A mutation was 14% (5 of 36 patients with BrS). After pooling all studies together, the mean prevalence rate of SCN5A mutations in 146 patients with BrS in the Chinese Han population was 7.5% (range: 0–25%, 7.5 ± 10.5%). The prevalence rate of SCN5A mutations in patients with BrS in Taiwan was close to the average (8.5% vs. 7.5%).

Discussion

In 1992, Pedro and Josep Brugada originally described the syndrome with a special ECG pattern consisting of an RBBB and at least 1-mm ST segment elevation in leads V1–V3 in association with SCD.1 Although BrS is prevalent worldwide and the exact prevalence is changing overtime, it is more common in Southeast Asian countries. In our previous study, the prevalence rate of Brugada-type ECG in a hospital-based population in Taiwan was 0.13%.22

BrS is generally considered to be a disorder in young male adults, with arrhythmogenic manifestation first occurring at an average age of 40 years.20 The male preference (86%), mean age of diagnosis, and clinical presenting symptoms of BrS in Taiwan were similar to those in

Table 1 Clinical characteristics of 14 patients with Brugada syndrome and SCN5A genetic variants.

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Sex</th>
<th>Age at diagnosis (y)</th>
<th>Presentation</th>
<th>Circumstance</th>
<th>Documented arrhythmias</th>
<th>Family history of SCD</th>
<th>Type of SCN5A variants</th>
<th>Type of Brugada ECG</th>
<th>ICD Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>47</td>
<td>Syncope/SCD</td>
<td>Awake at rest</td>
<td>VF</td>
<td>Mutation</td>
<td>Type I</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>34</td>
<td>Syncope</td>
<td>Awake at rest</td>
<td>N/A</td>
<td>Mutation</td>
<td>Type I</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>34</td>
<td>Dizziness</td>
<td>Awake at rest</td>
<td>Inducible VF</td>
<td>Mutation</td>
<td>Type II</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>48</td>
<td>Chest pain</td>
<td>Awake at rest</td>
<td>Inducible VF</td>
<td>Mutation</td>
<td>Type I</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>36</td>
<td>Syncope</td>
<td>Walking</td>
<td>VF</td>
<td>Positive SNP</td>
<td>Type I</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>50</td>
<td>Seizure</td>
<td>Sleeping</td>
<td>N/A</td>
<td>Positive SNP</td>
<td>Type I</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>24</td>
<td>Seizure</td>
<td>Awake at rest</td>
<td>VT</td>
<td>Positive SNP</td>
<td>Type I</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>70</td>
<td>Near syncope</td>
<td>Awake at rest</td>
<td>N/A</td>
<td>SNP</td>
<td>Type I</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>18</td>
<td>Syncope</td>
<td>Walking</td>
<td>N/A</td>
<td>SNP</td>
<td>Type II</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>24</td>
<td>SCD</td>
<td>Awake at rest</td>
<td>N/A</td>
<td>SNP</td>
<td>Type I</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>38</td>
<td>Chest</td>
<td>Awake at rest</td>
<td>N/A</td>
<td>SNP</td>
<td>Type I</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>44</td>
<td>SCD</td>
<td>Having lunch</td>
<td>VT</td>
<td>SNP</td>
<td>Types I and II</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>45</td>
<td>Syncope/</td>
<td>Sleeping</td>
<td>VF/VT</td>
<td>SNP</td>
<td>Type I</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>seizure/SCD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>M</td>
<td>51</td>
<td>Chest</td>
<td>Having dinner</td>
<td>N/A</td>
<td>SNP</td>
<td>Type I</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

ECG = electrocardiogram; ICD = implantable cardioverter-defibrillator; SCD = sudden cardiac death; SNP = single nucleotide polymorphism; VF = ventricular fibrillation; VT = ventricular tachycardia.

Table 2 Genetic results of Brugada syndrome patients with SCN5A variants.

<table>
<thead>
<tr>
<th>Number of unrelated individuals</th>
<th>Region</th>
<th>Nucleotide change</th>
<th>Coding effect</th>
<th>Location</th>
<th>Type of variations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Exon 12</td>
<td>1651 G&gt;A</td>
<td>Ala551Thr</td>
<td>DI/DII</td>
<td>Missense mutation</td>
</tr>
<tr>
<td>4</td>
<td>Exon 12</td>
<td>1673 A&gt;G</td>
<td>His558Arg</td>
<td>DI/DII</td>
<td>SNP</td>
</tr>
<tr>
<td>1</td>
<td>Exon 12</td>
<td>1776 C&gt;G</td>
<td>Asn592Lys</td>
<td>DI/DII</td>
<td>Missense mutation</td>
</tr>
<tr>
<td>6</td>
<td>Exon 18</td>
<td>3269 C&gt;T</td>
<td>Pro1090Leu</td>
<td>DII/DIII</td>
<td>SNP</td>
</tr>
<tr>
<td>2</td>
<td>Exon 20</td>
<td>3578 G&gt;A</td>
<td>Arg1193Gln</td>
<td>DII/DIII</td>
<td>Missense mutation</td>
</tr>
</tbody>
</table>

D = domain; SNP = single nucleotide polymorphism.

* Relative to c: NM_00109405.1.
Caucasian populations. In contrast, the index event of BrS in Taiwan occurred mostly when patients were awake at rest rather than sleeping. Only two of the 14 patients with BrS (14%) experienced the index event in the early morning when they were sleeping. Although BrS is an inheritable arrhythmic disease having an autosomal dominant pattern with incomplete penetrance, only three of the 14 BrS patients with SCN5A variants had a family history of SCD. This implied that patients with BrS in Taiwan have a preponderance of sporadic cases than that of familial cases.

In 1998, Chen et al first reported that BrS has a genetic basis that is linked only to mutations in SCN5A, the gene that encodes the β-subunit of the sodium channel. After more than 10 years, the SCN5A gene is still the most common BrS-causing gene and is responsible for 20–25% of this disease in Caucasian populations and for as much as 40% in cases of familial BrS. In 2011, the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA) expert consensus statement described that comprehensive SCN5A-targeted BrS genetic testing can be useful for any patient in whom a cardiologist has established a clinical index of suspicion for BrS based on examination of the patient’s clinical history, family history, and expressed electrocardiographic (resting 12-lead ECGs and/or provocative drug challenge testing) phenotype. However, the prevalence of SCN5A mutations in patients with BrS in the Chinese Han population in Taiwan remains unknown. This is the first study to report the result of SCN5A genetic testing for BrS in the largest cohort in Taiwan. We found that the prevalence of SCN5A mutations for BrS in the Chinese Han population in Taiwan was less than half of that reported in Caucasian populations (8% vs. 20–25%, p = 0.0007), but was close to the average prevalence of SCN5A mutations in pooling 143 patients with BrS in the Chinese Han population from several studies (8.5% vs. 7.5%, p = 0.83). This demonstrated that the ethnic difference plays an important role on BrS and certainly affects the genetic results. It is plausible that the SCN5A gene may not be the major BrS-causing gene in the Chinese Han population as that is in Caucasian populations.
Several characteristics of SCN5A mutations in our patients with BrS were different from those in other racial populations. First, a retrospective analysis of BrS databases showed that 293 mutations in SCN5A have been described in association with BrS across most white and a few nonwhite patients. We found three missense mutations, including two novel mutations (A551T and N592K), in our patients with BrS. Second, the four most frequent BrS-associated mutations were E1784K, F861WfsX90, D356N, and G1408R in the international compendium of SCN5A mutations for BrS. However, we did not detect any of them in our patients with BrS. Third, of the 293 unique mutations, 208 (71%) localized to one of the four transmembrane-spanning regions (DI, DII, DIII, or DIV), 54 (18%) localized to an interdomain linker, 17 (6%) localized to the C terminus, and 14 (5%) localized to the N terminus. The three mutations and two nonsynonymous SNPs we identified were interdomain linkers located in DI/II or DII/III separately instead of transmembrane-spanning regions.

Although several differences existed between the genetic characteristics of SCN5A mutations in patients with BrS in Taiwan and that in Caucasians populations, the two populations were similar in some aspects. First, 225 (77%) of the 293 mutations reported worldwide were identified only once. The two novel mutations we found were also identified once in our patients with BrS. In addition, of the 438 SCN5A mutation-positive cases, only 13 (3%) harbored multiple mutations. In addition, in our study, none of the patients with BrS had multiple SCN5A mutations. The genetic results demonstrated that single point mutation was the main genetic presentation in BrS in both Caucasian and Asian populations.

In this study, we found that 10 patients with BrS have nonsynonymous SNPs (1673 A>G or 3269 C>T) in the SCN5A gene. We found that 1673 A>G SNP was not significantly associated with an increased risk of BrS among the different inheritance models of analyses (OR = 1.9, p = 0.23 in additive model; OR = 2.3, p = 0.17 in the autosomal dominant model), whereas 3269 C>T SNP was significantly associated with BrS (OR = 18, p < 0.001) in both the additive and autosomal dominant model. The results suggested that patients with BrS in Taiwan are more likely to have the 3269 C>T SNP rather than 1673 A>G SNP compared with healthy controls (Supplementary Tables 1 and 2). This 3269 C>T SNP requires further functional studies.

In conclusion, compared with Caucasian populations, the patients with BrS in Taiwan had some different clinical characteristics and low prevalence of SCN5A mutations. In 2011, the HRS and EHRA expert consensus statement announced that mutation-specific genetic testing is recommended for family members and appropriate relatives following the identification of the BrS-causative mutation in an index case. Given that SCN5A remains the most common BrS genotype despite accounting for 8% of BrS in Taiwan, genetic heterogeneity of the disease is evident. Recently, 10 minor BrS-susceptibility genes (GPD1L, CACNA1C, CACNB2B, CACNA2D1, SCN1B, SCN3B, MOG1, KCND3, KCNE3, and KCNE5) were discovered and should be screened for in Chinese Han patients with BrS but without SCN5A genetic variants.
Limitations

The first limitation is that although this is the largest cohort of patients with BrS in Taiwan, some patients in other hospitals were not referred for genetic tests because of family matters or inconvenient accesses to our genetic core laboratory. However, BrS is a rare genetic disease. Approximately, three-fourth of patients with BrS in this cohort was referred from other medical centers or hospitals in Taiwan in the past 10 years. With the support, this cohort has become the largest referral cohort of patients with BrS in Taiwan. In addition, the geographic distributions of the referred patients with BrS were from the north, south, east, and west of Taiwan. As a result, this BrS cohort may represent the domestic genetic pattern of BrS in Taiwan. The study result could be an important genetic reference for BrS in Taiwan. The second limitation is that we did not screen microstructural DNA variants such as copy number variations on the SCN5A gene that warrant further studies.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jfma.2013.02.002.

References