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ORIGINAL ARTICLE

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

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Background/Purpose: Brugada syndrome (BrS) is a hereditable 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.

Conflicts of interest: The authors have no conflicts of interest relevant to this article.

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Results: SCN5A genetic variants were identified in 14 of the 47 patients with BrS and four of the 14 patients with BrS had missense mutations (1651 G>A, 1776 C>G, 3578 G>A). The prevalence rate of *SCN5A* mutations was approximately 8% (4/47), which was significantly lower than that reported in Caucasian populations (20–25%; $p = 0.0007$). The average age of these 14 BrS patients with *SCN5A* variants at diagnosis (12 men and 2 women) was 40 ± 13 years. Four patients experienced SCD, and six presented with seizure or syncope. Only three patients (3/14, 21.4%) had a family history of SCD.
Conclusion: The prevalence of *SCN5A* mutations in the Chinese Han population in Taiwan may be lower than that reported in the Caucasian populations. In addition, most patients with BrS did not have a family history of SCD.

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 V_1, V_2, and V_3 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.[2,3] 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.[4,5]

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).[6] The average age at the time of initial diagnosis or sudden death is 46 ± 7 years, especially in men in our previous report.[7] 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[8] 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 (V_1–V_3) 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

the buffy 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 supernates 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 ddH_2O.

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 of 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 $MgCl_2$ 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).[21] Mutations or single nucleotide polymorphisms (SNPs) within all 27 translated exons, slice sites, and exon–intron boundaries of the *SCN5A*-encoded cardiac sodium channel $Na_V1.5$ were detected using direct sequencing. Five hundred unrelated and ostensibly healthy volunteers with no clinical cardiac arrhythmias and with normal ECGs were genotyped as a control group. Mutations were defined as rare, case-only (absent in the 500 healthy volunteers enrolled from the Chinese Han population in Taiwan) variants with amino acid change that are possibly pathogenic. Variants with a minor allele frequency >0.5% among the 500 healthy volunteers in the Chinese Han population in Taiwan were termed as SNPs.

Statistical analysis

All continuous data were expressed as mean ± standard deviation. A frequency comparison was performed using the Chi-square test. Confidence intervals (CIs) of the binominal distribution were calculated for the prevalence rates. To compare the genetic effect of *SCN5A* SNP on BrS patients with healthy controls, we analyzed the distribution of allele and genotype frequencies in the two groups and calculated the odds ratio (OR) using different inheritance models including additive model, autosomal dominant model, and autosomal recessive model. Hardy–Weinberg equilibrium of allele and genotype distribution of *SCN5A* polymorphisms were tested using a Chi-square test. We used logistic regression to calculate OR of increased risk for BrS for each SNP. A two-tailed $p < 0.05$ was considered significant.

Results

Clinical characteristics of BrS patients with SCN5A genetic variants

SCN5A genetic variants were screened in all the 47 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).

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

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

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

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.[12–16] 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 V_1–V_3 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. It typically occurs with sudden death during sleeping.[20] The male preference (86%), mean age of diagnosis, and clinical presenting symptoms of BrS in Taiwan were similar to those in

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

Number of unrelated individuals	Region	Nucleotide change[a]	Coding effect	Location	Type of variations
1	Exon 12	1651 G>A	Ala551Thr	DI/DII	Missense mutation
4	Exon 12	1673 A>G	His558Arg	DI/DII	SNP
1	Exon 12	1776 C>G	Asn592Lys	DI/DII	Missense mutation
6	Exon 18	3269 C>T	Pro1090Leu	DII/DIII	SNP
2	Exon 20	3578 G>A	Arg1193Gln	DII/DIII	Missense mutation

D = domain; SNP = single nucleotide polymorphism.
[a] Relative to c: NM_00109405.1.

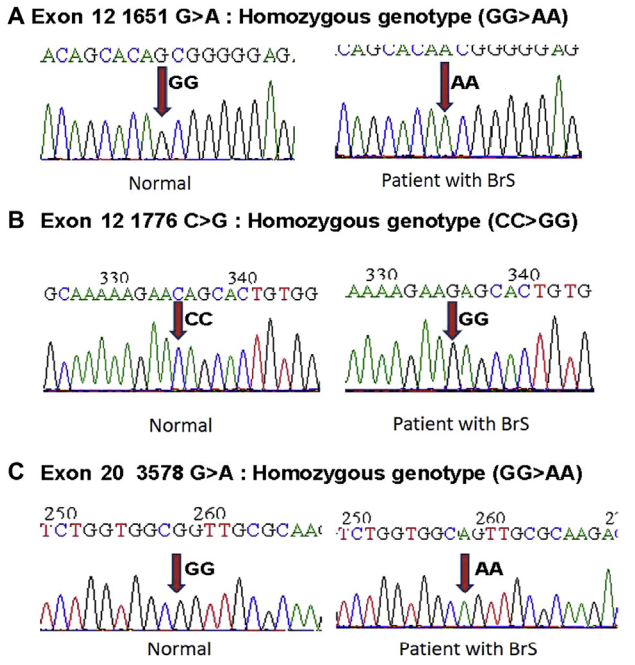

Figure 1 DNA sequencing results of three missense mutations identified on the *SCN5A* gene. (A) The arrows indicate two nucleotide changes from GG to AA transition (homozygous genotype) at position 1651 on exon 12 in a patient with Brugada syndrome (BrS). (B) The arrows indicate two nucleotide changes from CC to GG transition (homozygous genotype) at position 1776 on exon 12 in a patient with BrS. (C) The arrows indicate two nucleotide changes from GG to AA transition (homozygous genotype) at position 3578 on exon 20 in two patients with BrS.

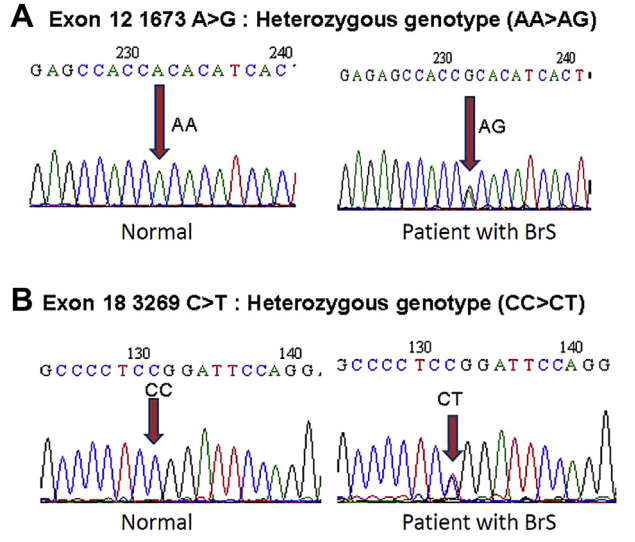

Figure 2 DNA sequencing results of two single nucleotide polymorphism identified on the *SCN5A* gene. (A) The arrows (two spikes with different colors) indicate a nucleotide change from AA to AG transition (heterozygous genotype) at position 1673 on exon 12 in four patients with Brugada syndrome (BrS). (B) The arrows (two spikes with different colors) indicate a nucleotide change from CC to CT transition (heterozygous genotype) at position 3269 on exon 18 in six patients with BrS.

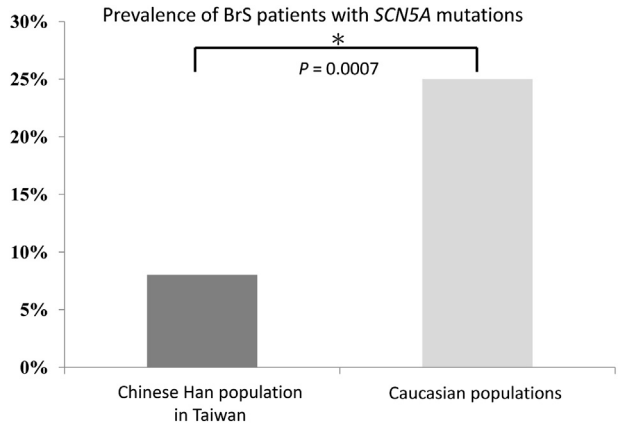

Figure 3 Significant difference in the prevalence of Brugada syndrome (BrS) patients with *SCN5A* mutations between the Chinese Han population in Taiwan and Caucasian populations.

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.[8] 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.[9,23] 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.[23] 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%, $p = 0.83$).[12–16] 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.

Table 3 Summary of studies on *SCN5A* mutations in patients with Brugada syndrome in the Chinese Han population.

Author	Location	Number of BrS patients	Number of BrS patients (proband) receiving genotyping	Number of symptomatic BrS patients	*SCN5A* positive in proband	*SCN5A* positive in symptomatic proband	Prevalence of *SCN5A* mutations	Number of controls	Reference
Mok et al	Hong Kong	50	36	20	5	2	14% (5/36)	400 white and 100 Chinese	12
Liang et al	Beijing	13	4	4	1	1	25% (1/4)	50 Chinese	13
Liang et al	Beijing	13	4	1	1	1	25% (1/4)	50 Chinese	16
Yuan et al	Nanjing	7	7	4	0	0	0%	0	14
Chen et al	Hangzhou	48	48	NA	0 (5 SNPs)	0	0%	120 Chinese	15
Juang et al	Taiwan	47	47	47	4	4	8.5% (4/47)	500 Chinese	Our study
Total		178	146		11		7.5% (11/146)		

BrS = Brugada syndrome; NA = not available; SNP = single nucleotide polymorphism.

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.[24] 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[25] 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.

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