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SHORT COMMUNICATION

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Genetic analysis of the cardiac sodium channel gene *SCN5A* in Koreans with Brugada syndrome

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Abstract The *SCN5A* gene encodes the alpha subunit of the human cardiac voltage-gated sodium channel. Mutations in *SCN5A* are responsible for Brugada syndrome, an inherited cardiac disease that leads to idiopathic ventricular fibrillation (IVF) and sudden death. In this study, we screened nine individuals from a single family and 12 sporadic patients who were clinically diagnosed with Brugada syndrome. Using PCR-SSCP, DHPLC, and DNA sequencing analysis, we identified a novel single missense mutation associated with Brugada syndrome in the family and detected a C5607T polymorphism in Korean subjects. A single nucleotide substitution of G to A at nucleotide position 3934 changed the coding sense of exon 21 of the *SCN5A* from glycine to serine (G1262S) in segment 2 of domain III (DIII-S2). Four individuals in the family carried the identical mutation in the *SCN5A* gene, but none of the 12 sporadic patients did. This mutation was not found in 150 unrelated normal individuals. This finding is the first report of a novel mutation in *SCN5A* associated with Brugada syndrome in Koreans.

Keywords Brugada syndrome · Ventricular fibrillation · *SCN5A* · Mutation · Koreans

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Introduction

Ventricular fibrillation (VF) is the most common cardiac arrhythmia and is usually present in heart-disease patients with structural cardiac abnormalities (Belhassen and Viskin 1993). A small subset of individuals with a structurally normal heart and no known etiology have a cardiac disorder that causes episodes of syncope or cardiac sudden death. These arrhythmias are classified as idiopathic ventricular fibrillation (IVF) (Belhassen and Viskin 1993). A subgroup of patients with IVF have a distinctive electrocardiogram (ECG) pattern with right bundle branch block (RBBB) and ST-segment elevation in leads V_1 to V_3 and a high incidence of sudden death (Brugada and Brugada 1992, 1997). The prevalence of IVF associated with this distinctive ECG pattern, which is commonly referred to as Brugada syndrome, accounts for 40–60% of all European IVF patients (Chen et al. 1998).

Brugada syndrome has been investigated worldwide and is also present in Asian populations, including Thais, Laotians, Cambodians, Vietnamese, Filipinos, Japanese, and Chinese, while no cases have been reported in Africans (Nademanee et al. 1997; Naccarelli and Antzelevitch 2001). Sudden unexplained death during sleep (SUDS) has been reported to be a leading cause of death among young men in northeastern Thailand, and about 40% of these victims have a family history of SUDS (Tatsanavivat et al. 1992). The mechanisms responsible for SUDS remain unclear.

In 1998, Brugada syndrome was shown to be a genetic disease with an autosomal dominant mode of

transmission (Chen et al. 1998). Mutations in *SCN5A*, the human cardiac voltage-gated sodium channel alpha subunit gene, have been associated with cardiac conduction disease (Keating and Sanguinetti 2001), chromosome 3-linked long-QT syndrome (LQT3), and Brugada syndrome (Wang et al. 1995; Chen et al. 1998). Channel proteins are responsible for the initial phase of the action potential in most excitable cells. Heterologously expressed Brugada syndrome mutant sodium channels have various severe functional abnormalities in their activation and fast inactivation gating properties, all of which result in reduced Na^+ channel capability (Akai et al. 2000; Keating and Sanguinetti 2001).

The *SCN5A* gene is located on human chromosome 3p21 and is only expressed in human heart tissue. The gene is comprised of 28 exons and encodes a protein of 2,016 amino acids with a molecular mass of 227 kDa (Gellens et al. 1992). Brugada syndrome was initially thought to have a high penetrance, but recent reports have revealed that the penetrance may in fact be as low as 20% (Priori et al. 2000; Hiraoka 2003). Furthermore, no *SCN5A* mutations have been found in some Brugada syndrome patients, suggesting the possibility of genetic heterogeneity (Priori et al. 2000). Many *SCN5A* mutations have been documented in patients with Brugada syndrome in a number of ethnic groups. While some of the mutations in the *SCN5A* gene lead to changes in the biophysical characteristics of the sodium channel, i.e., a reduction in the fast sodium channel current, the others are unlikely to cause functional alterations in the channel (Chen et al. 1998; Iwasa et al. 2000; Baroudi et al. 2002).

In this study, we screened in a family of nine individuals as well as 12 sporadic Korean patients to identify Brugada-associated mutations. We report here a novel missense mutation, G1262S (exon 21), in the *SCN5A* gene, which causes a nonsynonymous amino acid substitution in segment 2 of domain III (DIII-S2).

Subjects and methods

Subjects

We studied a family with nine members as well as 12 unrelated sporadic patients, all Koreans, diagnosed with Brugada syndrome. Patients and control subjects, all of whom provided informed consent, were recruited from the Yonsei Cardiovascular Center, Seoul, South Korea. Brugada syndrome is defined as a cardiac abnormality with no structural heart disorder. Diagnosis is based on ECG characteristics consisting of at least a 2-mm ST-segment elevation in more than one right precordial lead (V_1 to V_3) with an RBBB morphology as well as ventricular fibrillation resulting in sudden cardiac death (SCD) (Brugada and Brugada 1992). An implantable cardioverter defibrillator (ICD) was implanted in all patients. The control samples consisted of 150 (300 alleles) unrelated healthy Koreans with no cardiac symptoms.

Mutation analysis

Genomic DNA was prepared from peripheral blood leukocytes using QIAamp DNA blood mini kits (Qiagen, Valencia, CA, USA). All 28 exons of the *SCN5A* gene were amplified by polymerase chain reaction (PCR) using the modified intronic primer sequences and PCR conditions described by Wang et al. (1996). PCR products were analyzed by single-strand conformation polymorphism (SSCP) analysis, denaturing high-performance liquid chromatography (DHPLC), and DNA sequencing. The SSCP analyses were performed using 4–20% gradient NOVEX polyacrylamide TBE gels (Invitrogen, Carlsbad, CA, USA). PCR products were denatured for 5 min at 98°C in 0.4 M methylmercuric hydroxide (Boehringer Ingelheim, Heidelberg, Germany), which stabilizes denatured single-stranded DNA, placed in an ethanol ice bath for 3 min, and then loaded onto the gel. The samples were separated at a constant 200 V at 4°C or 15°C using a NOVEX ThermoFlow electrophoresis system (Invitrogen). To increase the chances of detecting sequence variations, the PCR products for the SSCP analyses were kept at approximately 250 bp. Since exons 2, 12, 16, 17, and 28 were large, we designed primers to divide them into several fragments. The bands were visualized by silver staining.

DHPLC analysis was used to detect sequence variations in PCR products using the WAVE system (Transgenomic, Omaha, NE, USA) as described in a previous report (Underhill et al. 1997). DHPLC analysis was performed at 60°C as calculated by the WAVE Maker software. For the efficient analysis of exon 21, a modified forward primer (GC-clamp; 5′-GCGTCCCGTCCAGGCTTCATGTCCACCT-3′) was designed. This system was used to verify *SCN5A* mutations in 150 (300 alleles) control subjects. Aberrant SSCP conformers were isolated from the native polyacrylamide gel and were re-amplified. The PCR products were purified using the QIAquick PCR purification kit (Qiagen) and sequenced using the ABI PRISM big dye terminator cycle sequencing ready reaction kit and ABI PRISM 3100 DNA sequencer (Applied Biosystems, Foster City, CA, USA). All the *SCN5A* exons of family members with an abnormal pattern as well as those from one normal control subject were sequenced. Sequences were compared with previously reported *SCN5A* cDNA sequences using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). Numbering of *SCN5A* was based on hH1 (GenBank accession no. M77235) and IHGSC (GenBank accession no. AC137587) for the consensus reference sequences.

Results

All patients, including the proband of the family, had Brugada-type ECG patterns (Fig. 1) consisting of RBBB and ST-segment elevation in leads V_1–V_3. The proband, a 52-year-old man (II-1, Fig. 2A), was referred to our institution because of recurrent episodes of palpitation. Programmed electrical stimulation easily induced atrial flutter. The application of radiofrequency energy at the inferior vena cava-tricuspid annulus (IVC-TA) isthmus successfully created bidirectional isthmic conduction block and eliminated common atrial flutter. Because he had Brugada pattern ECG abnormality, the induction study for ventricular tachycardia (VT) was carried out, and polymorphic VT was induced by triple ventricular extrastimulation. Familial history revealed that his father (I-1) had died of SCD during sleep at the age of 56 years. ECG recordings from the proband revealed dynamic ECG changes. The panel (c) represented coved ST-segment elevation (type 1), while panel (a) and (b) showed a typical type 2 ECG pattern (Fig. 1A). The proband also had symptomatic sinus node dysfunction and an abnormally prolonged QT interval (QTc) of 444 ms. The conduction parameters, i.e., PR interval and QRS duration, were 284 ms and 124 ms, respectively. An ICD and the pacemaker were implanted to prevent SCD and sinus node dysfunction. The ECG recording of an affected brother (II-3) showed typical type 2 ST-segment elevation (Fig. 1B).

Sporadic patient KB1 had experienced a loss of consciousness and inducible VT. There was no defined family history of SCD. The ECG had a typical J wave and type 1 ST-segment elevation in leads V_2 with an RBBB pattern. The ICD records showed several episodes of ventricular fibrillation. Patient KB2 had several episodes of aborted SCD and was diagnosed with Brugada syndrome. The ECG showed type 1 ST-segment elevation. During the electrophysiological study, VF was induced. Patient KB3 had several episodes of syncope and aborted SCD. The ECG pattern showed typical type 1 ST-segment elevation with an RBBB pattern. Patient KB4 had several episodes of syncope and type 3 ST-segment elevation (saddleback and ST-segment elevation <1 mm). The rest of the patients displayed characteristic ECG patterns and clinical events, including syncope and cardiac arrest in the absence of structural heart disease.

To investigate the genetic cause of Brugada syndrome in Korean patients, we screened the entire translated region of *SCN5A*. PCR-SSCP analysis revealed aberrant conformers for exon 21 in the family (Fig. 2B). This abnormal pattern was present in only four individuals in the family. DNA sequencing analysis of this aberrant conformer revealed a G-to-A base substitution at nucleotide position 3934 in exon 21 of *SCN5A* (Fig. 2C) that led to the replacement of glycine by serine at codon

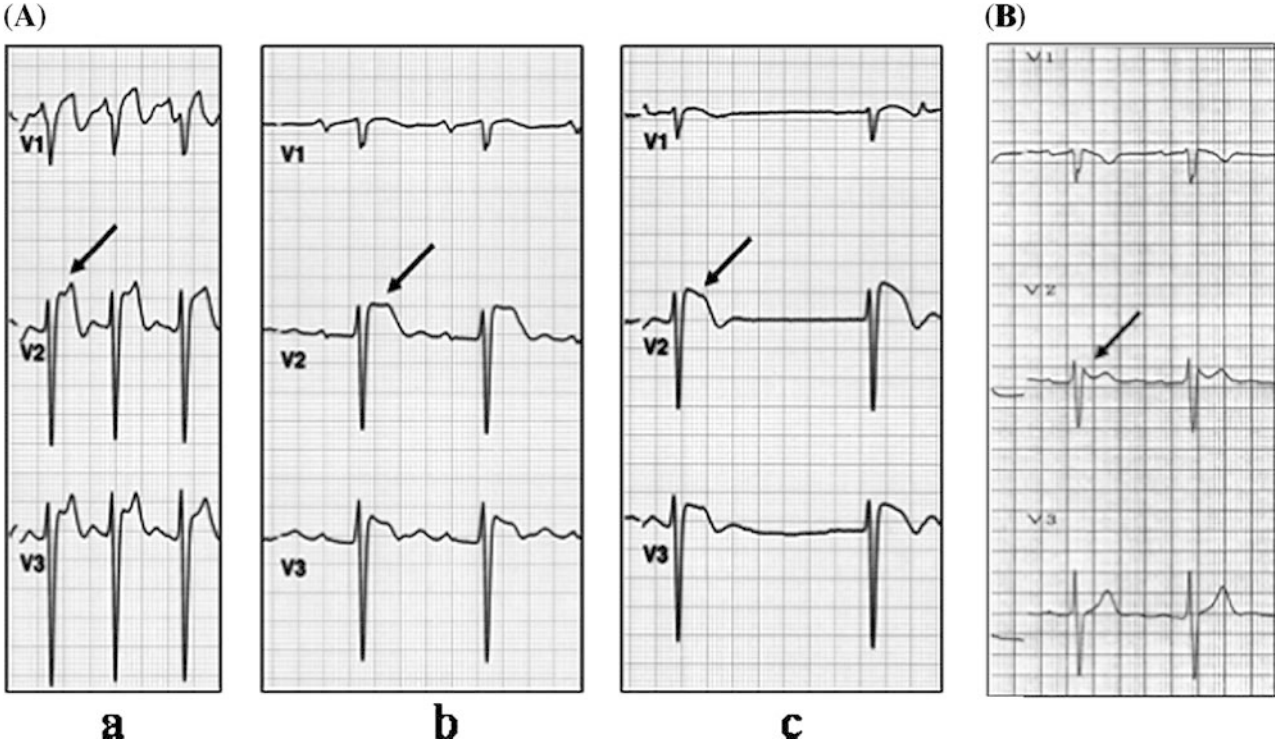

Fig. 1A, B Precordial leads V_1–V_3 of patients in a Korean family with Brugada syndrome. **A** ECG recordings of a proband. Note the dynamic ECG changes with no drug-challenge test in the course of a month, and two patterns are shown. *Panel a,b* show clear type 2 ECG pattern and *panel c* shows type 1. **B** Representative recording of the ECG of an affected family member (II-3). ECG pattern shows the typical type 2 Brugada syndrome phenotype. *Arrow* in each panel indicates the J-wave

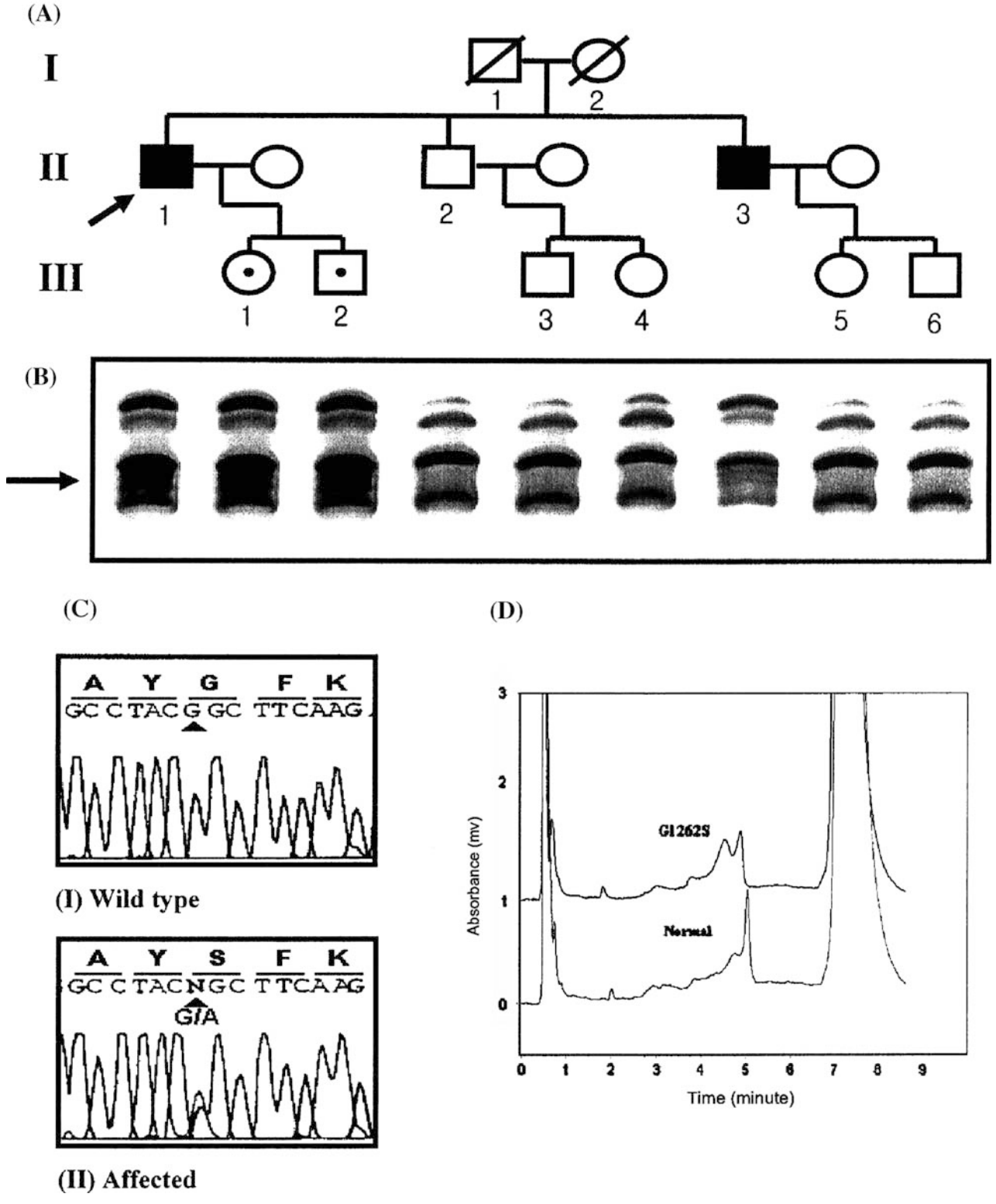

Fig. 2A–D Mutational screening of the *SCN5A* gene in Korean family KBSF1. **A** Partial pedigree structure and phenotypic identification of the family. The *arrow* indicates the proband. The *open circles* and *squares* represent normal females and males, respectively. Affected subjects are represented by *solid symbols*. **B** The results of the PCR-SSCP analysis of exon 21 of *SCN5A* are shown below the pedigree. Aberrant SSCP conformers are indicated by *arrows*. **C** DNA sequencing analysis of exon 21 from a normal control subject (I) and an affected patient (II). The DNA sequence shows a missense mutation (G-to-A substitution) at nucleotide position 3934, which results in an amino acid substitution of glycine by serine at codon 1262 (G1262S). **D** DHPLC confirmed an abnormal peak in two affected individuals (II-1 and II-3) and two descendants (III-1 and 2) of the proband as well as 150 unrelated control individuals

1262 (G1262S) at the terminal region of transmembrane segment 2 of domain III (DIIIS2) of the human cardiac sodium channel. The nonsynonymous nucleotide substitution G3934A (G1262S) was present as a heterozygous state in four affected individuals, including the proband in the family. No other *SCN5A* mutations were found in these subjects. This mutation was not detected by DHPLC in the other family members, in the 12 unrelated sporadic patients, nor in the 150 unrelated control individuals (Fig. 2D). Based on the sequences of the coding regions of the *SCN5A* gene, one brother (II-2) in the family did not have the specific nonsynonymous nucleotide substitution for Brugada syndrome, although his ECG pattern presented type 2 ST-segment elevation (data not shown).

DNA sequence analysis also revealed a nucleotide change C5607T resulting in a synonymous substitution (D1819D). This variation, which has been reported in the American, Japanese, and Han Chinese populations (Wattanasirichaigoon et al. 1999; Iwasa et al. 2000; Takahata et al. 2003; Chen et al. 2004), was present with 0.685 and 0.315 frequencies for C and T alleles, respectively, in the general Korean population.

Discussion

We studied genetic variations in the *SCN5A* gene of Korean patients with Brugada syndrome and normal control subjects. Clinical diagnosis of the patients was confirmed by ECG findings such as an RBBB pattern and ST-segment elevation in leads V_1 through V_3 with no distinct structural cardiac abnormalities. The clinical histories of the patients were consistent with previous reports (Brugada and Brugada 1992, 1997).

To date, several groups have demonstrated that Brugada syndrome results from mutations in the *SCN5A* gene (Chen et al. 1998; Priori et al. 2000; Takahata et al. 2003). In the family with Brugada syndrome that we studied, we identified a novel missense mutation, G1262S, in the S2 transmembrane segment in domain III of *SCN5A*. Four family members were shown to carry the identical mutation in the *SCN5A* gene. No other mutations were found in the gene. In addition, this mutation was absent in 300 alleles in unaffected normal individuals. The mutation detected in our study was unique to this family. The variation we discovered was thus a mutation, not a polymorphism.

Genetic screening of exon 21 revealed that segregation of the mutation had occurred in this family. The proband (II-1) had a typical Brugada-type ECG pattern and carried the heterozygous G1262S allele. The proband's descendants (III-1 and III-2) had the same heterozygous mutant allele. However, they did not have a history of clinical symptoms, and an ECG was not performed for these individuals due to their young ages (6 and 4 years old, respectively). Patient II-3 also had the heterozygous allele but did not transmit any mutant alleles to his descendants (III-5 and III-6).

Most of the *SCN5A* mutations that have been investigated in Brugada patients are on the linker segments (P-loop). These mutations mostly cause either an acceleration of the recovery of the Na^+ channel or result in a nonconducting Na^+ channel. The functional effects of mutations in the transmembrane domains, including G1262S, have not yet been documented. The incidence of *SCN5A* mutations in Brugada syndrome differ among ethnic groups and families, as previously reported (Priori et al. 2000; Hiroaka 2003). In addition, the mutational variability infers that other genes linked to this disorder or other factors that modulate the phenotype may contribute to cardiac events, although none have yet been identified. Recent reports have revealed that some Brugada syndrome patients do not have mutations in the *SCN5A* gene (Smits et al. 2002; Takahata et al. 2003). This is consistent with our results, which suggest that some patients diagnosed with Brugada syndrome carry patient-specific mutations in *SCN5A*. On the other hand, patients who lack a unique mutation would have other mutations that cause Brugada syndrome.

In our study, we showed that there was a novel mutation (G1262S) in the *SCN5A* gene in Korean patients with Brugada syndrome and a synonymous polymorphism (D1819D) in the Korean population. In a future study, we will conduct a functional analysis of the mutations using a mammalian expression system, which may help clarify the pathogenic involvement of these substitutions. We will also attempt to identify additional mutation sites in other genes that may be responsible for Brugada syndrome.

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