The Spectrum of SCN5A Gene Mutations in Spanish Brugada Syndrome Patients

Mónica García-Castro,ª Cristina García,ª Julián R. Reguero,ª Ana Miar,ª José M. Rubín,ª Victoria Álvarez,ª César Morís,ª and Eliecer Cotoª,ª,ª

ªGenética Molecular, Hospital Universitario Central de Asturias, Oviedo, Asturias, Spain
ªServicio de Cardiología, Fundación Asturcor, Hospital Universitario Central de Asturias, Oviedo, Asturias, Spain
ªInstituto de Investigación Nefrológica-Fundación Renal Íñigo Álvarez de Toledo, Madrid, Spain

INTRODUCTION

Brugada syndrome is characterized by right bundle branch block and ST-segment elevation in the right precordial ECG leads. Familial transmission is frequent and approximately 25% of cases exhibit mutations in the SCN5A gene. We analyzed the sequence of this gene in 25 Spanish patients with Brugada syndrome. In 4 (16%), we found mutations that had not previously been described: three were amino acid changes (i.e. Ala2>Thr, Ala735>Thr and Val1340>Ile) and one was an intron mutation that affected messenger RNA processing (i.e. IVS18-1G>A). These four patients had relatives who were also mutation carriers, several of whom had normal ECGs, even on flecainide challenge. Our study suggests that genetic analysis could be helpful in the presymptomatic diagnosis of Brugada syndrome, but may be less useful for stratifying the risk of adverse events.

Key words: Brugada syndrome. SCN5A gene. Mutations. Genetic risk.

Espectro mutacional del gen SCN5A en pacientes españoles con síndrome de Brugada

El síndrome de Brugada se caracteriza por un bloqueo de la rama derecha y elevación del segmento ST en las derivaciones precordiales derechas del electrocardiograma. Con frecuencia se observa una transmisión familiar, y en aproximadamente el 25% de los casos se han hallado mutaciones en el gen SCN5A. Hemos analizado la secuencia de este gen en 25 pacientes españoles con síndrome de Brugada. En 4 de ellos (16%) hallamos mutaciones que no habían sido descritas previamente: 3 eran cambios de aminoácidos (Ala2>Tre, Ala735>Tre y Val1340>Ile) y 1 era intrónica y afectaría al procesamiento del ARNm (intrón 18 IVS18-1G>A). En los 4 había familiares portadores, y varios de ellos tenían electrocardiogramas normales, incluso tras inducción con flecainida. Nuestro estudio indica que el análisis genético sería útil para el diagnóstico presintomático, pero de utilidad limitada para estratificar el riesgo de eventos adversos.

Palabras clave: Síndrome de Brugada. Gen SCN5A. Mutaciones. Riesgo genético.
in patients with Brugada syndrome, as well as in some cases of type-3 long-QT syndrome (LQT3), Lev-Lenègre syndrome, and atrial fibrillation. All of these mutations cause a reduction in sodium channel function. Recent reports have suggested that Brugada syndrome may also be associated with mutations in other genes, such as glycerol-3-phosphate dehydrogenase (GPD1L) and the alpha-1 and beta subunits of the L-type cardiac calcium channel (CACNA1C and CACNB2b). It remains unclear what proportion of patients without mutations in SCN5A carry mutations in one of these other genes.

The aim of this study was to characterize the array of SCN5A mutations in a group of Spanish patients and define the phenotype associated with the mutations identified.

METHODS

Patients

The study included 25 patients diagnosed by specialists from the Department of Cardiology at Hospital Universitario Central de Asturias. Diagnosis was based on the presence of a type-1 ECG with ST-segment elevation followed by a negative T wave. Patients with type-1 or -2 ECG were diagnosed with Brugada syndrome if they had a type-1 pattern in more than 1 of the right precordial leads (V1-V3) after administration of flecainide. In addition, diagnostic workup included an electrophysiology study to assess inducibility of ventricular arrhythmias and measure conduction times. Table 1 shows the main characteristics of the patient population.

Genetic Study

Genomic DNA was obtained by extraction from leukocytes in 10 mL of peripheral blood. Polymerase chain reaction (PCR) was used to amplify the 27 coding exons from SCN5A with primers designed using the flanking intronic regions. Each fragment was purified and sequenced using BigDye chemistry in an ABI3130 sequencer (Applied Biosystems, Foster City, California, USA). The sequence for each patient was compared with the SCN5A sequence in the Ensembl sequence database (reference SCN5A ENSG00000183873; www.ensembl.org). Further information on the primers and PCR conditions can be obtained from the corresponding author.

Any sequence variant that had not been described previously was analyzed in 200 healthy individuals (ages, 20–70 years) recruited through the Principality of Asturias blood bank. None of these control subjects had symptoms of heart disease, although ECG was not performed to rule out arrhythmias. Each fragment in which a possible mutation had been identified was amplified in the patient and the 200 controls and the genotype defined by single-strand conformational polymorphism (SSCP). SSCP was also used to define the genotype of the H558R polymorphism in patients and controls.

In the cases in which a mutation in SCN5A was observed, all family members were offered a diagnostic workup including baseline ECG and screening for the mutation. Family members who were carriers of the mutation but had a normal ECG were studied further using the flecainide test. All individuals included in the study (patients, family members, and controls) provided signed informed consent to the genetic study.

RESULTS

Of the 25 patients included in the study, 18 (64%) had a type-1 baseline ECG and 7 (36%) had type 2 or 3 ECGs that were converted to type 1 in the flecainide test (Table 1). In those patients, we found 16 nucleotide variants of SCN5A, and only 4 of those were not also found in at least 1 of the corresponding 200 controls. None of the 4 mutations had been described previously in patients with Brugada syndrome (Table 2). The Ala2>Tre, Ala735>Tre, and Val1340>Ile mutations affected conserved amino acids. The 4 patients were homozygous for histidine 558; consequently, the effect of this polymorphism on the phenotype could not be assessed. Below we describe the main characteristics of the patients and their family members.

### TABLE 1. Characteristics of the 25 Patients with Brugada Syndrome Included in the Study

<table>
<thead>
<tr>
<th>Age at diagnosis, y</th>
<th>42 (14); range, 17-66</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men/women</td>
<td>18/7 (72%)</td>
</tr>
<tr>
<td>Age in men</td>
<td>40 (14) y; range, 17-66 y</td>
</tr>
<tr>
<td>Age in women</td>
<td>49 (11) y; range, 36-60 y</td>
</tr>
<tr>
<td>Baseline ECG</td>
<td></td>
</tr>
<tr>
<td>Type 1</td>
<td>18 (72%)</td>
</tr>
<tr>
<td>Type 2 or 3</td>
<td>7 (28%)</td>
</tr>
<tr>
<td>Family history of Brugada syndrome</td>
<td>5 (20%)</td>
</tr>
<tr>
<td>Family history of sudden death (&lt;45 y)</td>
<td>9 (36%)</td>
</tr>
<tr>
<td>Syncope</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>Nocturnal agonal respiration</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Electrophysiology study</td>
<td></td>
</tr>
<tr>
<td>VT</td>
<td>6 (24%)</td>
</tr>
<tr>
<td>NSVT</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>VF</td>
<td>1 (4%)</td>
</tr>
</tbody>
</table>

ECG indicates electrocardiogram; NSVT, nonsustained ventricular tachycardia; VF, ventricular fibrillation; VT, ventricular tachycardia.

*In the 7 patients with type-2 or -3 baseline ECG, type-1 ECG was induced with flecainide.
The electrophysiology study was negative. The patient and 4 family members were carriers of the mutation: mother (76 years), sister (52 years), and 2 sons (32 and 36 years). All had a normal ECG both at baseline and in the flecainide test.

**DISCUSSION**

In our study, 16% of patients carried mutations in SCN5A, a rate that is similar to previously reported results.\(^5,8\) As in other studies, we analyzed coding exons and some intronic bases. Consequently, we cannot rule out the possibility that mutations were present in other regions of the gene (such as the promoter or intronic sequences). Our study only analyzed SCN5A, but other genes have recently been described that could also be mutated (although less frequently) in patients with Brugada syndrome.\(^7,9\) Patients with mutations in these genes would have a shorter QT interval and a new clinical entity has therefore been proposed combining Brugada syndrome and short-QT syndrome.

The 4 mutations identified in our patients had not been described previously (see the Biobase:HGMD database, available from www.biobase-international.com). This suggests that direct analysis would not be effective for the identification of mutations and in most cases sequencing of SCN5A would be necessary. Although we have not undertaken studies to assess the functional consequences of the mutations identified here, it can be expected that they would lead to malfunction of the sodium channel and cardiac conduction abnormalities.

Only 2 of the patients (50%) had a family history of Brugada syndrome, and among the family members who were carriers of the mutation, many were asymptomatic and had normal ECG results. In the largest patient series that has been published (130 patients), Priori et al\(^5\) found that 23% of family members who also carried the mutation did not have symptoms of the disease. Only the coexistence of syncope and ST-segment elevation in the ECG would be an indicator of poor prognosis in these patients, since the mutations are not associated

---

**TABLE 2. Characteristics of the 4 Patients Carrying Mutations in SCN5A**

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Change</th>
<th>Carrier, Age/Sex</th>
<th>Family History of Sudden Death</th>
<th>Baseline ECG</th>
<th>Electrophysiology Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.4G&gt;A (exon 2)</td>
<td>Ala2Thr GCA&gt;ACA</td>
<td>52/male</td>
<td>No</td>
<td>Type 2(^a)</td>
<td>Negative</td>
</tr>
<tr>
<td>c.2203G&gt;A (exon 14)</td>
<td>Ala735Thr GCG&gt;ACG</td>
<td>60/female</td>
<td>No</td>
<td>Type 1</td>
<td>Negative</td>
</tr>
<tr>
<td>c.3390-1G&gt;A (intron 18)</td>
<td>c.3390-1G&gt;A</td>
<td>60/male</td>
<td>Yes</td>
<td>Type 1</td>
<td>Positive</td>
</tr>
<tr>
<td>c.4018G&gt;A (exon 23)</td>
<td>Val1340Ile GTC&gt;ATC</td>
<td>55/male</td>
<td>Yes</td>
<td>Type 2(^a)</td>
<td>Negative</td>
</tr>
</tbody>
</table>

ECG indicates electrocardiogram.

\(^a\) Type-1 ECG induced with flecainide.

---

**Ala2Thr (c.4G>A) Mutation**

The patient was a 52-year-old man with a family history of Brugada syndrome diagnosed during routine screening. He had a type-2 ECG, which was converted to type 1 by flecainide. The electrophysiology study was negative. His mother (75-year-old) was a carrier of the mutation but was asymptomatic and had a normal ECG both at baseline and following flecainide challenge.

**Ala735Thr (c.2203G>A) Mutation**

The patient was a 60-year-old woman with recurring palpitations and no family history of sudden death. She had a type-1 baseline ECG and the electrophysiology study was normal. The patient, a sister aged 64 years, and 2 daughters aged 29 and 28 years were carriers of the mutation. Her sister and younger daughter had a type-2 baseline ECG that was converted to type 1 following flecainide challenge. The elder daughter had a normal ECG both at baseline and following flecainide challenge.

**IVS18-1G>A (c.3390-1G>A) Mutation**

Male patient diagnosed at 40 years of age with a type-1 baseline ECG and a positive electrophysiology study. The patient’s mother and a son had died suddenly at 52 and 20 years of age, respectively. We found a mutation in the last nucleotide of intron 18 (G>A), and analysis using a computer program (http://www.fruitfly.org/seq_tools/other.html) showed that the mutation would affect RNA processing, eliminating exon 19 of the mRNA. This would represent a splicing mutation, and the patient’s 30-year-old daughter was also a carrier. The daughter had a normal baseline ECG that was converted to type 1 in the flecainide test.

**Val 1344Ile (c.4018G>A) Mutation**

The index case was a 55-year-old man with a type-1 ECG whose brother had died suddenly at age 45. The family history of Brugada syndrome was positive, and the patient was also a carrier of the mutation. He and his family members had a normal ECG both at baseline and following flecainide challenge.
worse prognosis. Those authors concluded that genetic data would serve as a diagnostic criterion but would be of little use in predicting the risk of adverse events.

Finally, some polymorphisms in SCN5A (such as H558R) could also be phenotypic modifiers in patients with mutations in this gene. The genotype of these polymorphisms may explain clinical or ECG differences among carriers of the mutation, even among family members.\(^\text{10}\) Since all of the carriers of mutations in SCN5A were homozygotes for histidine 558, we were unable to draw conclusions regarding the phenotypic effect of this polymorphism.

In conclusion, the frequency of SCN5A mutations in our patients was similar to that described in other populations. The existence of family members who were carriers of the mutation but remained asymptomatic and had a normal ECG indicates that genetic studies are of limited usefulness for determining risk but remain helpful for diagnosis.

**REFERENCES**