SCN5A Mutations and the Role of Genetic Background in the Pathophysiology of Brugada Syndrome

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Background—Mutations in SCN5A are identified in ≈20% to 30% of probands affected by Brugada syndrome (BrS). However, in familial studies, the relationship between SCN5A mutations and BrS remains poorly understood. The aim of this study was to investigate the association of SCN5A mutations and BrS in a group of large genotyped families.

Methods and Results—Families were included if at least 5 family members were carriers of the SCN5A mutation, which was identified in the proband. Thirteen large families composed of 115 mutation carriers were studied. The signature type I ECG was present in 54 mutation carriers (BrS-ECG+, 47%). In 5 families, we found 8 individuals affected by BrS but with a negative genotype (mutation-negative BrS-ECG+). Among these 8 mutation-negative BrS-ECG+ individuals, 3, belonging to 3 different families, had a spontaneous type I ECG, whereas 5 had a type I ECG only after the administration of sodium channel blockers. One of these 8 individuals had also experienced syncpe. Mutation carriers had, on average, longer PR and QRS intervals than noncarriers, demonstrating that these mutations exerted functional effects.

Conclusions—Our results suggest that SCN5A mutations are not directly causal to the occurrence of a BrS-ECG in the absence of gross structural abnormalities. However, in familial studies, the relationship between SCN5A mutations and BrS remains poorly understood. The aim of this study was to investigate the association of SCN5A mutations and BrS in a group of large genotyped families.

Key Words: death, sudden (if surviving, use heart arrest) ■ Brugada syndrome ■ SCN5A ■ genetics ■ tachyarrhythmias ■ arrhythmia

Brugada syndrome (BrS) is an inherited arrhythmia syndrome with an increased risk of sudden death, resulting from polymorphic ventricular tachycardia and/or ventricular fibrillation in the absence of gross structural abnormalities. BrS is associated with ST-segment elevation in the right precordial ECG leads, which have such a characteristic shape (so-called type I ECG, here abbreviated as “BrS-ECG+,” see Methods) that their presence is required for the diagnosis. A BrS-ECG+ may occur spontaneously or be provoked by sodium channel blocking drugs. Although BrS and a BrS-ECG+ are intimately linked, not all patients with a BrS-ECG+ are at risk of ventricular fibrillation, as individuals with a BrS-ECG+ exhibit marked phenotypic variability, ranging from sudden death victims to individuals in whom a BrS-ECG+ is found by chance, but who remain asymptomatic. The phenotypic variability has spawned studies aimed at finding modifying factors such as gender, age, and other environmental factors. Recent experimental studies support a role of the genetic background, although clinical observations indicate that the risk of sudden death of a BrS patient is not increased if otherwise unexplained sudden death has occurred in his/her family.

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Mutations in the SCN5A gene, which encodes the pore-forming subunit of the cardiac voltage-gated sodium channel, are found in 20% to 30% of BrS patients. Five other genes...
Table 1. Summary of the Phenotype-Genotype Relationship in the Study Group (n=13 Families)

<table>
<thead>
<tr>
<th>Family No.</th>
<th>Mutation (Nucleotide)</th>
<th>Mutation (Amino Acid)</th>
<th>Mutation Carriers</th>
<th>Mutation Positive BrS-ECG 1</th>
<th>Mutation Positive BrS-ECG 2</th>
<th>Mutation Positive, Phenotype Undetermined</th>
<th>Mutation Negative BrS-ECG 1</th>
<th>Type of Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>c.612–2A&gt;G</td>
<td>…</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>Wrong splice</td>
</tr>
<tr>
<td>B</td>
<td>c.4222G&gt;A</td>
<td>p.Gly1408Arg</td>
<td>14</td>
<td>4</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>Missense</td>
</tr>
<tr>
<td>C</td>
<td>c.673C&gt;T</td>
<td>p.Arg2257Trp</td>
<td>11</td>
<td>4</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>Missense</td>
</tr>
<tr>
<td>D</td>
<td>c.5164A&gt;G</td>
<td>p.Asn1722Asp</td>
<td>9</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>Missense</td>
</tr>
<tr>
<td>E</td>
<td>c.3963+2T&gt;C</td>
<td>…</td>
<td>10</td>
<td>2</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>Wrong splice</td>
</tr>
<tr>
<td>F</td>
<td>c.4145G&gt;T</td>
<td>p.Ser1382Ile</td>
<td>9</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>Missense</td>
</tr>
<tr>
<td>G</td>
<td>c.2516T&gt;C</td>
<td>p.Leu839Pro</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>Missense</td>
</tr>
<tr>
<td>H</td>
<td>c.2254G&gt;A</td>
<td>p.Gly752.Arg</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>Missense</td>
</tr>
<tr>
<td>J</td>
<td>c.1603C&gt;T</td>
<td>p.Arg535X</td>
<td>9</td>
<td>5</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>Non sense</td>
</tr>
<tr>
<td>K</td>
<td>c.3667delG</td>
<td>p.Ala1223ProfsX7</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>Frame shift, stop</td>
</tr>
<tr>
<td>L</td>
<td>p.1570–1571insilie</td>
<td>…</td>
<td>11</td>
<td>6</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>Insertion</td>
</tr>
<tr>
<td>M</td>
<td>c.481G&gt;A</td>
<td>p.Glu161Lys</td>
<td>11</td>
<td>7</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>Missense</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>115</td>
<td>54</td>
<td>55</td>
<td>6</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

(GPD1L, CACNA1C, CACNB2, SCN1B, and KCNE3) have been associated with BrS, but the prevalence of variants in these genes is yet unknown. SCN5A mutations may also lead to progressive cardiac conduction defects, long-QT syndrome type 3, or atrial standstill. Progressive cardiac conduction defects and BrS are both associated with a loss of function of the mutant sodium channel. Accordingly, “overlap” families who present a mixed phenotype with features of both diseases exist. The causality of SCN5A mutations in progressive cardiac conduction defects was proven by linkage analysis. In contrast, SCN5A mutations in BrS were discovered by a candidate gene approach and linkage data are still lacking, with the exception of a large overlap family in which the causal mutation is linked with a mixed phenotype. Of note, not only is the proportion of SCN5A-positive BrS families who did not carry the familial SCN5A mutation low, but also, conversely, BrS-ECGs+ were reported among members of SCN5A-positive BrS families who did not carry the familial SCN5A mutation. Clearly, the association between BrS and SCN5A mutations is complex. In this study, we set out to investigate this association in a larger group of families.

Methods

This retrospective study was conducted at the center of reference for rare arrhythmic diseases of Nantes University Hospital and at the Academic Medical Center, University of Amsterdam, in accordance with the local guidelines for genetic research and with the approval of the local medical ethics committees. Informed written consent was obtained from all patients.

Large genotyped BrS families in which 5 or more family members carried a SCN5A mutation were included for the present investigation. An individual was defined as affected by BrS if he/she displayed a BrS-ECG at baseline or after provocation with a class I antiarrhythmic drug (BrS-ECG+). A BrS-ECG+ was defined as a coved-type ST-segment elevation ≥0.2 mV at its peak followed (without isoelectric separation) by a negative T wave in 2 or more right precordial leads. Either intravenous ajmaline (1 mg/kg body weight at a rate of 10 mg/min) or flecainide (2 mg/kg body weight in 10 minutes with a maximum of 150 mg) were used for drug testing. Drug challenge was not performed in patients younger than 16 years of age and in those showing severe conduction defects at rest. Underlying structural heart disease was excluded by echocardiography, chest roentgenogram, and exercise testing. Laboratory tests excluded acute ischemia and metabolic or electrolyte disturbances.

ECG parameters that were analyzed, before and after drug testing, were as follows: heart rate, PQ interval (in lead II), QRS duration, maximal ST elevation (right precordial leads), and QTc duration in V4 (Bazett’s formula).

Genetic Analysis

Genomic DNA was extracted from peripheral blood leukocytes using standard protocols. All 28 exons of SCN5A were amplified by polymerase chain reaction using intronic primers. Polymerase chain reaction products were screened for a SCN5A mutation using denaturing high performance liquid chromatography-DNA sequencing. DNA variants were disease-causing mutations, rather than polymorphisms, if they were present in highly conserved regions of SCN5A and absent in 200 control individuals. Annotation of mutations was based on the cDNA reference sequence GenBank NM_198056. Sequencing of the SCN5A gene was performed in probands and in all mutation-negative BrS-ECG+ patients.

Statistical Analysis

ECG parameters were compared with Student t test. Clinical data are expressed as mean value±SD for continuous data or proportions for categorical data. A probability value <0.05 was considered significant.

Results

Clinical and Electrocardiographic Results

Among a total of 444 genotyped probands with BrS-ECGs+ in whom molecular screening was conducted in our international multicenter database, 118 (26%) carried an SCN5A mutation. Thirteen of them, each with a different mutation (Table 1), belonged to large families with ≥5 SCN5A-positive members. For this analysis, all the screened members of these 13 families were included, and they constituted the study population (n=264; Table 2). A history of sudden cardiac death at young age was present in 3 families. In the 13 probands, the workup that led to the diagnosis of BrS was conducted because of (1) unexplained syncopal episode...
(n=4); (2) ECG performed because of chest pain (n=2); (3) ECG performed because of palpitations (n=2); (4) ECG performed for other reason (n=4); and (5) ECG performed because of a family history of sudden death (n=1). Eleven of 13 probands had a spontaneous BrS-ECG (type I), whereas 1 showed a BrS-ECG only after drug provocation (family D; Figure 1). The last proband (family L) suffered from sudden cardiac death during car racing; his brother also died during car racing from sudden cardiac death and that second event initiated familial screening.

Genetic analysis of the probands’ relatives identified a total of 115 carriers (Figure 1) of the familial SCN5A mutation and 149 mutation negative relatives (mutation negative unaffected individuals not shown in Figure 1). In total, a spontaneous BrS-ECG+ was present in 21 of 115 mutation carriers (18%) and in 3 of the noncarriers. Provocation testing, performed in 67 mutation carriers and 63 noncarriers, was positive in 33 additional mutation carriers and in 5 noncarriers. Thus, among carriers of the familial mutation the penetrance of the BrS phenotype increased from 18% at baseline to 61% after drug testing (54/88 patients). Twenty-five mutation carriers received an implantable cardioverter/defibrillator.

At baseline, mutation carriers had, on average, significantly longer PR (193±37 ms versus 162±29 ms, \( P<0.00001 \)) and QRS intervals (113±20 versus 95±14 ms, \( P<0.0001 \)), compared with the noncarriers (Table 2). There was a higher proportion of patients affected by a first-degree AV block among carriers (27/115, 23%) than among noncarriers (4/149, 3%).

### Table 2. PR and QRS Duration in the 13 Families (264 Members)

<table>
<thead>
<tr>
<th>Status</th>
<th>n</th>
<th>PR Interval, ms</th>
<th>QRS Interval, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation positive BrS-ECG+</td>
<td>54</td>
<td>194±37</td>
<td>113±18</td>
</tr>
<tr>
<td>Mutation positive not BrS-ECG+</td>
<td>61</td>
<td>193±37</td>
<td>113±21</td>
</tr>
<tr>
<td>Mutation carriers (total)</td>
<td>115</td>
<td>193±37*</td>
<td>113±20**</td>
</tr>
<tr>
<td>Mutation negative BrS-ECG+</td>
<td>8</td>
<td>158±25</td>
<td>94±15</td>
</tr>
<tr>
<td>Mutation negative not BrS-ECG+</td>
<td>141</td>
<td>162±31</td>
<td>94±14</td>
</tr>
<tr>
<td>Mutation negative subjects (total)</td>
<td>149</td>
<td>162±29*</td>
<td>95±14**</td>
</tr>
</tbody>
</table>

\( *P<0.00001; \; **P<0.001 \)

Figure 1. Pedigrees of the 13 SCN5A-related BrS families. Squares represent males and circles represent females. Probands are indicated by an arrow. Family members affected by the BrS phenotype (BrS-ECG+) before or after provocation challenge are represented with a left-half filled symbol. Family members affected by progressive cardiac conduction disease are represented with a right half-filled symbol. Mutation carriers without BrS phenotype (mutation negative BrS-ECG−) are in white. Patients with undetermined phenotype are shown with gray symbol and unknown genotype is indicated by a “?” symbol. Family members carriers of a SCN5A mutation are indicated with + and noncarriers with −. The noncarriers of the SCN5A mutation but affected by the BrS are circled (mutation negative BrS-ECG−). Patients who underwent sodium channel blocker testing are indicated with a T. Unaffected mutation-negative individuals are not shown.
Results in the Families With Individuals Who Had a BrS-ECG+ but Did Not Carry the Familial Mutation (Mutation Negative BrS-ECG+)

In 5 of the 13 studied families, 8 individuals showed a BrS-ECG+, but did not carry the familial mutation (mutation negative BrS-ECG+ individuals; Figure 2). Among them, 3 (each from a different family) had a spontaneous BrS-ECG+, whereas 5 had a BrS-ECG+ only after drug testing. One was symptomatic (syncpe; d2), whereas the other 7 were asymptomatic (3 men and 4 women). Drugs or factors leading to acquired Br-ECG and carriership of a SCN5A mutation.

These 5 families were composed of 44 mutation-positive and 61 mutation-negative subjects in total (mutation-negative BrS subjects not shown in Figure 1). Also in this subgroup, mutation-positive individuals had, on average, significantly longer PR intervals (180±32 ms versus 158±25 ms, P=0.001) and QRS durations (107±18 ms versus 94±15 ms, P<0.0001) than mutation-negative individuals. Among the noncarriers of these families, there were no significant differences in conduction parameters between the subjects showing a BrS ECG and the ones without ST segment elevation (PR: 165±18 ms versus 157±26 ms, P=0.3; QRS: 98±14 ms versus 94±15 ms, P=0.4).

An electrophysiological study was performed in 5 BrS-ECG nonmutation patients. The mean HV interval was 50 ms. Ventricular tachyarrhythmias were inducible in 3 (1 with a spontaneous BrS-ECG and 2 with a BrS-ECG induced by ajmaline). Signal-averaged ECG was performed in all the mutation negative BrS-ECG+ patients and was negative in all. An implantable cardioverter/defibrillator was implanted in these 3 patients. No arrhythmic event was registered during a 52±42 months follow-up period.

Genetic Analysis in the Mutation Negative BrS-ECG+ Patients

To make sure that the BrS phenotype identified in these patients was not related to another mutation of the SCN5A gene, we have performed a complete sequencing of the SCN5A gene. This sequencing failed to identify any mutation.

Discussion

We set out to investigate the association between the occurrence of a BrS-ECG+ and carriership of a SCN5A mutation. For this purpose, we studied 13 families with BrS-ECGs+ in whom at least 5 family members carried the familial SCN5A mutation. We found that this association is complex, because BrS-ECGs+ were only found in 18% of mutation carriers at baseline and in 61% after drug testing, and conversely, 8 individuals had a BrS-ECG, but did not carry the familial mutation.

These observations may suggest that carriership of a SCN5A mutation is not causal to the occurrence of a BrS-ECG+. Historically, the putative role of SCN5A mutations in BrS is derived from the identification of loss-of-function mutations in many BrS syndrome patients throughout the world.20 In general, affected families are small, and genetic linkage data are lacking, with exception of a large family with an overlap syndrome where a sodium channel mutation cosegregated with a complex phenotype.21 If gene-negative, phenotype-positive patients (as present in 5 of these 13 families) would have been found in the initial years of the genotyping efforts in this disease entity, a causal role for SCN5A would have been probably refuted. Biophysical (patch-clamp) studies of the aberrant gene products (cardiac sodium channels) have consistently demonstrated that their altered functional properties are compatible with the BrS phenotype.32 All BrS-associated SCN5A mutants reported at present exhibit net loss-of-function which is generally regarded as the inciting pathophysiological mechanism of BrS (yet, whether reduced sodium channel function acts by exacerbating spatial inhomogeneities in depolarization or in repolarization to cause the signature ST elevations of BrS is still controversial).29 Among mutations found in our 13 families (Table 1), 2 mutations introduce wrong splice sites (c.612 to 2A and c.5963+2T>C), 2 are frameshift mutations (c.3816delG and c.1983 to 1993dup), 1 mutation introduces a premature stop codon (p.Arg553X), and 1 mutation causes an in-frame insertion (p.1570- Phe1571insIle). These mutations are all likely to produce nonfunctional sodium channels as usually identified in the BrS. The 7 remaining mutations are missense mutations, 4 of which have been biophysically studied (p.Glu161Lys23,30 p.Gly752Arg27,30 p.Gly1408Arg28 and p.Arg225Trp29) and have shown to produce a strong decrease in peak sodium current. Although no functional data are available for the remaining 3 missense mutations (p.Ser1382Ile, p.Leu839Pro, and p.Asn1722Asp), we found that mutation carriers have, on average, longer PR intervals and QRS durations than noncarriers. These findings are consistent with previous reports17,30 and suggest that the mutant channels produce less sodium current, as sodium current reduction causes cardiac conduction slowing. Thus,
the apparent lack of association between carriahership of a SCN5A variant and a BrS-ECG+, as observed in our study population, cannot be explained by the fact that these SCN5A variants are no disease-causing mutations. There is also no reason to believe that the subjects with BrS-ECG+, who do not carry the familial SCN5A mutation, have no true BrS. Indeed, among the genotype-negative phenotype-positive patients, 3 in 3 different families had a spontaneous BrS-ECG+, whereas 5 had a BrS-ECG+ only after sodium channel blockers. We have found no other clinical abnormalities to explain the electrocardiographic aspect of BrS. One of these individuals was symptomatic with syncope before the study. Finally, the electrophysiological study induced ventricular fibrillation in 3 of these 5 patients, as previously reported in BrS.\textsuperscript{3,4,35}

A novel notion to emerge from our observation that some patients have a BrS-ECG+, but do not carry the familial SCN5A mutation, is that modifying factors within the studied families (genetic background) are sufficiently powerful to evoke a BrS-ECG+. These observations are in line with emerging experimental studies, which indicate that disparate genetic backgrounds confer disparate susceptibilities to the effects of a single loss-of-function mutant sodium channel.\textsuperscript{6} Clearly, the relevant elements in the genetic background may encompass all proteins and molecules that play a role in the pathophysiology of BrS. For instance, such genes may include not only those that encode ion channel subunits but also those that encode molecules that modify cardiac structure (eg, fibrosis), thereby facilitating arrhythmias. Hence, it seems conceivable that the genetic background in some families is so strongly conducive to the occurrence of a BrS-ECG+ that a BrS-ECG+ occurs even in the absence of a SCN5A mutation or drug provocation (spontaneous BrS-ECG+). This might point to the fact that a loss-of-function SCN5A mutation, on its own, might not be sufficient to cause BrS but could act like a revelatory factor as sodium channel blocker challenge.

Limitations
All the genes known to be involved in the occurrence of the BrS have not been sequenced. Then it is possible that other gene mutations are responsible for the Brugada within the families presented in the study. A provocation testing has not been performed in all the family members, and then it is possible that we have underestimated the frequency of the BrS within these families.

Conclusion
We provide novel clinical evidence to suggest that genetic background may play a powerful role in the pathophysiology of BrS. These findings add further complexity to concepts regarding the causes of BrS and are consistent with the emerging notion that the pathophysiology of BrS includes various elements beyond mutant sodium channels that produce reduced sodium current.

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Disclosures
None.

References
This study focused on the complexity of the genetic background in Brugada syndrome (BrS). Mutations in SCN5A are present in ≈ 20% to 30% of probands affected by BrS. However, in familial studies, the relationship between SCN5A mutations and BrS remains to be demonstrated. In this investigation, Probst et al investigated the relationship between the presence of SCN5A mutations and BrS ECG in 13 families in which the proband and at least 4 other family members were carriers of SCN5A mutations. The authors demonstrated that the penetrance of the BrS is low. We also confirmed that mutation carriers had, on average, longer PR and QRS intervals than noncarriers, demonstrating that these mutations exerted functional effects. The major finding of this investigation is that in 5 of these 13 families, 8 family members affected by BrS were not carriers of the SCN5A mutation. These observations suggest that SCN5A mutations probably act as a major modulating factor revealing the syndrome, but it is likely that other factors like the genetic background play an important role in the occurrence of the disease. Overall, these results indicate that BrS is not a monogenic mendelian disease but probably an oligogenic disease. The low penetrance of the disease and the presence of so-called phenocopies demonstrated that there may not be a direct link between the presence of the SCN5A mutations and BrS. These results have to be taken in account for the genetic counseling of the patients.
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