The clinical and electrophysiological spectrum of cardiac sodium channel mutations
Smits, J.P.P.

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CHAPTER 3.3

GENOTYPE-PHENOTYPE RELATIONSHIP IN BRUGADA SYNDROME: ELECTROCARDIOGRAPHIC FEATURES DIFFERENTIATE SCN5A-RELATED PATIENTS FROM NON-SCN5A RELATED PATIENTS

Jeroen P. P. Smits MD*, Lars Eckardt MD†, Vincent Probst MD‡, Connie R. Bezzina PhD*, Jean Jacques Schott PhD‡, Carol Ann Remme MD*, Wilhelm Haverkamp MD†, Günter Breithardt MD†, Denis Escande MD, PhD‡, Eric Schulze-Bahr MD†, Hervé LeMarec MD, PhD‡, Arthur A. M. Wilde MD, PhD*

*Experimental and Molecular Cardiology Group, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands, †Medizinische Klinik und Poliklinik C (Kardiologie/Angiologie) Universitätsklinikum, Institut f. Arterioskleroseforschung an der Universität Münster, Münster, Germany, ‡INSERM U533, Hôpital Hôtel-Dieu, Nantes, France

Genotype-phenotype relationship in Brugada syndrome: electrocardiographic features differentiate SCN5A-related patients from non-SCN5A-related patients


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3.3.1 Abstract

Objectives: We have tested whether a genotype-phenotype relationship exists in Brugada syndrome (BS) by trying to distinguish BS patients with (carriers) and those without (non-carriers) a mutation in the gene encoding the cardiac sodium channel (SCN5A) using clinical parameters.

Background: Brugada syndrome is an inherited cardiac disease characterized by a varying degree of ST-segment elevation in the right precordial leads and (non)specific conduction disorders. In a minority of patients, SCN5A mutations can be found. Genetic heterogeneity has been demonstrated, but other causally related genes await identification. If a genotype-phenotype relationship exists, this might facilitate screening.

Methods: In a multi-center study, we have collected data on demographics, clinical history, family history, electrocardiogram (ECG) parameters, His to ventricle interval (HV), and ECG parameters after pharmacologic challenge with INa blocking drugs for BS patients with (n = 23), or those without (n = 54), an identified SCN5A mutation.

Results: No differences were found in demographics, clinical history, or family history. Carriers had a significantly longer PQ interval on the baseline ECG and a significantly longer HV time. A PQ interval of 210 ms and an HV interval 60 ms seem to be predictive for the presence of an SCN5A mutation. After INa blocking drugs, carriers had significantly longer PQ and QRS intervals and more increase in QRS duration.

Conclusions: We observed significantly longer conduction intervals on baseline ECG in patients with established SCN5A mutations (PQ and HV interval and, upon class I drugs, more QRS increase). These results concur with the observed loss of function of mutated BS-related sodium channels. Brugada syndrome patients with, and those without, an SCN5A mutation can be differentiated by phenotypical differences.

Abbreviations: AUC; area under the curve; BS; Brugada syndrome; ECG; electrocardiogram/electrocardiographic; EPS; electrophysiologic studies; HR; heart rate; HV; His to ventricle interval; Na⁺; sodium; SCN5A; pore-forming region (alpha-subunit) of the human cardiac sodium channel; SSCP; single-strand conformation polymorphism
3.3.2 Introduction
Brugada syndrome (BS) presents with malignant ventricular arrhythmias occurring in the structurally normal heart, leading to (aborted) sudden death. The hallmark electrocardiographic (ECG) feature of the disorder is ST-segment elevation in the right precordial leads.\(^1\)\(^-\)\(^3\) This ECG feature may not, however, be consistently present, and transient normalization of the ST segment is commonly observed.\(^3\)\(^,\)\(^4\) Other characteristic ECG features include a terminal negative T-wave in the right precordial leads and specific (i.e., left anterior hemiblock, right bundle branch block) or non-specific (QRS widening) conduction disorders. This phenotype is increasingly recognized as an inherited trait and is classified among the familial primary electrical disorders.\(^5\)

Mutations in the gene encoding the pore-forming subunit of the cardiac sodium channel, SCN5A, have been causally linked in patients and families with BS.\(^6\)\(^,\)\(^7\) In an effort to clarify the underlying pathophysiology, several mutated sodium channels associated with the disorder have been studied in heterologous expression systems. In all cases, a reduction in sodium (Na\(^+\))-current amplitude (I\(_{\text{Na}}\)), either by a reduction in Na\(^+\) channel expression or through alterations in channel gating properties,\(^7\) is predicted. Clinically, a reduced Na\(^+\) current is expected to impact on conduction properties.

An SCN5A mutation, however, is found only in a minority (~15\%) of patients.\(^8\) Genetic heterogeneity is also evidenced by linkage to a region on chromosome 3 (3p22-25), other than the SCN5A locus (3p21), in a family with the disorder.\(^9\)

Genetic heterogeneity also underlies the different sub-types of the inherited long QT syndrome, in which the various forms are associated with mutations in multiple cardiac ion channel genes. In this disorder, clinical and ECG features display genotype-specific characteristics. Apart from shedding light on the pathophysiologic mechanisms of the various sub-types, this facilitates genetic screening,\(^10\) permits faster genetic testing, and thereby provides opportunities for earlier initiation of optimal patient management. It is unclear whether such a genotype-phenotype relationship exists in BS. In this study, we have tested whether it is possible to distinguish between SCN5A-mutation carriers and patients without a sodium channel mutation on the basis of clinical and ECG criteria.

3.3.3 Methods
Patient population
Patients diagnosed with BS at the following three university hospitals were included: Academic Medical Center, Amsterdam (The Netherlands); the University of Münster,
Genotype-phenotype relationship in Brugada syndrome: electrocardiographic features differentiate SCN5A-related patients from non-SCN5A-related patients

Münster (Germany) and Hôpital Hôtel-Dieu, Nantes (France). All tests that were performed were approved by the medical ethical review committees of the hospitals involved. Informed consent was obtained from all patients.

Clinical data, including age at diagnosis, gender, (non)pharmacologic therapy and family history, were obtained retrospectively from patient records from the respective hospitals. Patients without a suspected index event but with a malignant family history were diagnosed with BS based on the presence of ECG characteristics consisting of at least 2-mm ST-segment elevation in leads V1 to V3 and (in)complete right bundle branch block morphology. In patients with aborted cardiac arrest, 1 mm was sufficient. Underlying (structural) heart disease was excluded by echocardiography, cardiac catheterization, chest roentgenograms, and exercise testing. In addition, laboratory tests to exclude (acute) ischemia and metabolic or electrolyte disturbances were performed. In total, 77 patients were studied, all of whom were genetically tested for the presence of an SCN5A mutation. In order to avoid the predominant effect of any single mutation, only index patients from each family (and no other affected family members) were included.

**ECG, electrophysiologic measurements, and sodium channel blocking drugs**

The first 12-lead ECG available in the absence of drugs was analyzed. Heart rate (HR), PQ, QRS and QT intervals were measured. The QT interval was corrected using Bazett's formula (QTc = QT/RR). The maximal ST-segment elevation in leads V1 to V3 was measured.

Baseline electrophysiologic studies (EPS) were performed in 46 (eight SCN5A-related and 38 non-SCN5A-related) of 77 patients. The His to ventricle interval (HV) and inducibility of ventricular arrhythmias, using up to two extra stimuli, were registered.

The effect of Na\(^+\) channel blocking drugs was tested in 53 (11 SCN5A-related and 42 non-SCN5A-related) of 77 patients. In three patients, pre-existing ECG abnormalities were considered too severe for safe testing of these drugs. Two patients refused the test.

The choice of drug was determined by the availability of the drug in the hospitals concerned. In 28 patients, ajmaline (1 mg/kg body weight intravenous at a rate of 10 mg/min) was used; in 23 patients, flecainide (2 mg/kg body weight intravenous in 10 min with a maximum of 150 mg) was used; and in two patients, procainamide (10 mg/kg intravenous at a rate of 100 mg/min) was used. The same ECG measurements as before were performed after administration of the sodium channel blocking drugs.

**Mutation analysis of SCN5A**

In all patients, all 28 exons of SCN5A were amplified by polymerase chain reaction from DNA isolated from peripheral leukocytes, utilizing intronic primers. Polymerase chain
reaction products were subjected to single-strand conformation polymorphism (SSCP) analysis followed by direct sequence analysis of aberrant conformers.\textsuperscript{11} In order to increase the probability of detecting the presence of any sequence change, SSCP was carried out at two different temperatures for each exon, and the size of fragments for SSCP was kept around 300 base pairs.\textsuperscript{12,13}

**Statistical analysis**

The Student $t$ test or Mann-Whitney test was performed, where appropriate, to test for statistical differences between two mean values. A paired $t$ test was used for the evaluation of changes in ECG parameters before and after sodium channel blocking drugs, with $p$ values of $<0.05$ considered statistically significant. Proportional differences between groups were analyzed using the Fisher exact test. Sensitivity, specificity, and receiver-operator curves were computed to select possible cut points in the measured values and test for the diagnostic utility of these.

### 3.3.4 Results

**Patient population and mutation analysis of SCN5A**

The population consisted of a total of 77 patients, 23 SCN5A-related (Table 1) and 54 non-SCN5A-related patients, respectively. Patient characteristics are summarized in Table 2. The male:female ratio was not significantly different between the two groups. Within the whole group, the age at diagnosis was on average eight years younger in males compared with females: 45.1 ± 11.2 years versus 53.0 ± 11.7 years ($p < 0.05$). Among SCN5A-mutation carriers, males were on average 13 years younger than females: 40.9 ± 9 years versus 53.6 ± 6 years ($p < 0.05$). Because no further significant differences between males and females regarding index event and ECG parameters could be found, these groups were not studied separately in subsequent analyses. No significant differences in age, family history, and index event were observed between mutation carriers and non-carriers (Table 2).

**ECG parameters and electrophysiologic measurements**

The SCN5A-related patients showed a longer PQ interval on their baseline 12-lead ECG compared with non-SCN5A-related patients (209 ± 51 ms vs. 163 ± 23 ms [$p < 0.0001$]; Table 2; Fig. 1A). No significant differences were observed in HR, QRS duration, QTc interval and ST-segment elevation (Table 2).

Patients with a mutation in SCN5A were found to have a significantly longer HV interval, as measured during EPS (66 ± 13 ms), than patients without an SCN5A mutation (48 ± 9 ms [$p < 0.001$]) (Table 2; Fig. 1B). Ventricular fibrillation or polymorphic ventricular tachycardia
was induced during programmed electrophysiologic stimulation in the majority of both SCN5A-related (6 of 8, 75%) and non-SCN5A-related patients (27 of 38; 71%) who underwent EPS (p = NS). No difference between the HV interval of symptomatic and asymptomatic patients was observed (data not shown).

Table 1. Included SCN5A Mutations
From two families two symptomatic index persons were included. Two other mutations were identified in two unrelated families (proven by haplotype analysis).

<table>
<thead>
<tr>
<th></th>
<th>Brugada Patients With SCN5A Mutation</th>
<th>Brugada Patients Without SCN5A Mutation</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (n)</td>
<td>23</td>
<td>54</td>
<td>—</td>
</tr>
<tr>
<td>Gender (m/f)</td>
<td>15/8</td>
<td>44/10</td>
<td>NS</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>45 ± 15</td>
<td>48 ± 11</td>
<td>NS</td>
</tr>
<tr>
<td>Family history +</td>
<td>11/23 (48%)</td>
<td>24/54 (44%)</td>
<td>NS</td>
</tr>
<tr>
<td>Index event</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VF/VT</td>
<td>6/23 (26%)</td>
<td>15/54 (28%)</td>
<td>NS</td>
</tr>
<tr>
<td>Syncope</td>
<td>8/23 (35%)</td>
<td>19/54 (35%)</td>
<td>NS</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>7/23 (30%)</td>
<td>15/54 (28%)</td>
<td>NS</td>
</tr>
<tr>
<td>Others*</td>
<td>2/23 (9%)</td>
<td>5/54 (9%)</td>
<td>NS</td>
</tr>
<tr>
<td>Baseline ECG (n)</td>
<td>23</td>
<td>54</td>
<td>—</td>
</tr>
<tr>
<td>Heart rate (beats/min⁻¹)</td>
<td>67 ± 15</td>
<td>71 ± 12</td>
<td>NS</td>
</tr>
<tr>
<td>PQ interval (ms)</td>
<td>209 ± 51</td>
<td>163 ± 24</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>QRS duration (ms)</td>
<td>110 ± 24</td>
<td>102 ± 22</td>
<td>NS</td>
</tr>
<tr>
<td>QTc interval (ms⁻²)</td>
<td>405 ± 31</td>
<td>400 ± 35</td>
<td>NS</td>
</tr>
<tr>
<td>ST-segment elevation (mm)</td>
<td>2.5 ± 1.3</td>
<td>1.9 ± 1.3</td>
<td>NS</td>
</tr>
<tr>
<td>Electrophysiologic testing (n)</td>
<td>8</td>
<td>38</td>
<td>—</td>
</tr>
<tr>
<td>VT/VF inducible</td>
<td>6/8 (75%)</td>
<td>27/38 (71%)</td>
<td>NS</td>
</tr>
<tr>
<td>HV time</td>
<td>66 ± 13</td>
<td>48 ± 9</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 2. Characteristics of Patients With or Without SCN5A Mutation
ECG = electrocardiogram; HV = His to ventricle interval; SCN5A = pore-forming region of the human cardiac sodium channel; VF = ventricular fibrillation; VT = ventricular tachycardia.

Sodium channel blocking drugs
Both within the total tested patient population (n = 51 [excluding the 2 patients who were tested using procainamide]) as within the non-SCN5A-related patient group (n = 41), no
significant differences between ajmaline and flecainide could be observed. Within the SCN5A-related patient group, six patients received flecainide, and four received ajmaline. Also, no difference could be observed. Procainamide was used in one SCN5A-related and one non–SCN5A-related patient. Because previous authors have also reported that they could not find significant differences in effects of these drugs on ECG parameters, we did not distinguish between the different drugs in our analysis. No differences in basic patient characteristics (ECG, index event, and so forth) were observed between patients challenged and patients not challenged.

In SCN5A-related patients, Na\(^+\) channel blocking drugs elicited a significant increase in PQ-interval, QRS-duration, and ST-segment elevation (Table 3). Similarly, in non–SCN5A-related patients, administration of Na\(^+\) channel blocking drugs caused a significant increase in PQ interval, QRS duration, and ST-segment elevation (Table 3). In addition, in these patients, HR and QTc interval were also found to be increased (Table 3).

After administration of Na\(^+\) channel blocking drugs, the difference in PQ interval between SCN5A-related and non–SCN5A-related patients was maintained; SCN5A-related patients had a significantly longer PQ interval than non–SCN5A-related patients (222 ± 37 ms vs. 195 ± 33 ms [p < 0.05]). In addition, a difference in QRS duration became evident between SCN5A-related and non–SCN5A-related patients after drug administration; SCN5A-related patients had a significantly longer QRS interval than non–SCN5A-related patients (142 ± 31 ms vs. 118 ± 21 ms [p < 0.05]) (Table 3). No significant differences between the two groups were observed regarding changes in HR, QTc-interval, and ST-segment elevation (Table 3).

Interestingly, when comparing the effects of Na\(^+\) channel blockade between SCN5A-related and non–SCN5A-related patients, a significantly larger increase in QRS duration in the former group was observed (38 ± 31 ms vs.18 ± 18 ms [p < 0.05]) (Table 3).

### 3.3.5 Discussion

In this study we investigated whether a genotype-phenotype relationship exists in BS. Probing this issue is of interest because, if such a correlation exists, it might facilitate future genetic screening in patients with the disorder.

Several mutations linked to BS have been described in SCN5A, the only gene hitherto linked to the disorder. In vitro studies on implicated mutations have consistently shown a reduction in Na\(^+\) current either by a decrease in cell-surface expression or by a reduction of unitary current secondary to altered biophysical properties. This points toward the likely involvement of altered Na-current characteristics in the generation of both the ST-segment
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elevation and ventricular arrhythmias. Decreased net inward (or an increased net outward) current at the end of phase 1 of the cardiac action potential is thought to underlie epicardial action potential shortening by aggravating the natural difference in repolarization characteristics of endocardium and epicardium, with resultant ST-segment elevation in the leads overlying the respective area. The resultant electrical heterogeneity in action potential between epicardial and endocardial layers is expected to be arrhythmogenic.

<table>
<thead>
<tr>
<th>ECG before class I antiarrhythmic drug</th>
<th>Brugada Patients With SCN5A Mutation (n) 11</th>
<th>Brugada Patients Without SCN5A Mutation (n) 42</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beats/min⁻¹)</td>
<td>65 ± 16</td>
<td>72 ± 12*</td>
<td>NS</td>
</tr>
<tr>
<td>PQ interval (ms)</td>
<td>195 ± 40*</td>
<td>164 ± 27†</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>QRS duration (ms)</td>
<td>104 ± 26†</td>
<td>100 ± 17‡</td>
<td>NS</td>
</tr>
<tr>
<td>QTc interval (ms/10²)</td>
<td>402 ± 28</td>
<td>405 ± 34‡</td>
<td>NS</td>
</tr>
<tr>
<td>ST-segment elevation (mm)</td>
<td>2.3 ± 1.6†</td>
<td>1.8 ± 1.0‡</td>
<td>NS</td>
</tr>
<tr>
<td>ECG after class I antiarrhythmic drug</td>
<td>Brugada Patients With SCN5A Mutation (n) 11</td>
<td>Brugada Patients Without SCN5A Mutation (n) 42</td>
<td>p Value</td>
</tr>
<tr>
<td>HR (beats/min⁻¹)</td>
<td>72 ± 17</td>
<td>76 ± 13*</td>
<td>NS</td>
</tr>
<tr>
<td>PQ interval (ms)</td>
<td>222 ± 37*</td>
<td>195 ± 33‡</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>QRS duration (ms)</td>
<td>142 ± 31†</td>
<td>118 ± 21‡</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>QTc interval (ms/10²)</td>
<td>426 ± 60</td>
<td>431 ± 40‡</td>
<td>NS</td>
</tr>
<tr>
<td>ST-segment elevation (mm)</td>
<td>4.5 ± 1.1†</td>
<td>3.9 ± 1.2‡</td>
<td>NS</td>
</tr>
<tr>
<td>Difference in ECG parameters after class I antiarrhythmic drug</td>
<td>Brugada Patients With SCN5A Mutation (n) 11</td>
<td>Brugada Patients Without SCN5A Mutation (n) 42</td>
<td>p Value</td>
</tr>
<tr>
<td>ΔHR (beats/min⁻¹)</td>
<td>10 ± 15</td>
<td>4 ± 12</td>
<td>NS</td>
</tr>
<tr>
<td>ΔPQ interval (ms)</td>
<td>26 ± 28</td>
<td>32 ± 21</td>
<td>NS</td>
</tr>
<tr>
<td>ΔQRS duration (ms)</td>
<td>38 ± 31</td>
<td>18 ± 18</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>ΔQTc interval (ms/10²)</td>
<td>23 ± 64</td>
<td>27 ± 40</td>
<td>NS</td>
</tr>
<tr>
<td>ΔST-segment elevation (mm)</td>
<td>2.2 ± 1.4</td>
<td>2.1 ± 1.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 3. ECG Before and After Class I Antiarrhythmic Drug Challenge
ECG = electrocardiogram; HR = heart rate; SCN5A = pore-forming region (alpha-subunit) of the human cardiac sodium channel.

The sodium current, however, not only impacts on action potential duration but also plays an important role in propagating the action potential. Hence, a reduction in Na⁺ current is also expected to negatively affect conduction within the heart. As a matter of fact, SCN5A mutations have also been shown to underlie familial conduction disease. It is therefore not unexpected that conduction disorders are found in BS patients with an SCN5A mutation. Indeed, the prolonged PQ interval at the baseline ECG observed in our SCN5A-mutation
 carriers (likely partially due to the significantly prolonged HV interval in these individuals) supports the role for Na channel in conduction.\textsuperscript{5} Also, though not statistically significant, QRS duration tended to be longer in SCN5A mutation carriers. Moreover, these ECG parameters are predictive for the presence or absence of an SCN5A mutation in BS patients (Figs. 1 and 2). When a PQ interval of 210 ms or more is considered to predict the presence of an SCN5A-related defect, a sensitivity of 48% and specificity of 98% is reached (Fig. 1A). In parallel, when an HV interval of 60 ms or more is considered to predict the presence of an SCN5A mutation, a sensitivity of 88% and a specificity of 82% are attained (Fig. 1B), with an area under the curve (AUC) of 0.788 for the baseline PQ duration (Fig. 2A) and 0.875 for the HV interval (Fig. 2B). These two parameters have a fair-to-good accuracy as a test for predicting the presence or absence of an SCN5A mutation within the patient group diagnosed with BS.

Administration of Na\textsuperscript{+} channel blocking drugs to BS patients in general (i.e., whether they are SCN5A-related or non–SCN5A-related patients) is expected to interfere with the ion current underlying phase 1 of the cardiac action potential, thereby exacerbating the ST-segment elevation. Patients without mutation in SCN5A may also develop conduction delay after challenge with Na\textsuperscript{+} channel blocking agents, as has been shown both for individuals with and individuals without BS.\textsuperscript{17,18} However, patients with an SCN5A mutation and, thus, preexistent reduced sodium current are expected to be the most susceptible to development (or further development) of conduction delay. Our finding, that SCN5A-mutation carriers show a larger increase in QRS duration after Na-channel blockade than non–SCN5A-related patients, confirms this hypothesis.

In addition to the PQ interval at baseline, the PQ and QRS intervals after Na-channel blockade are predictive of the presence or absence of an SCN5A mutation: both a PQ-interval of 225 ms and a QRS interval of 140 ms (abnormal prolongation in both cases) predict the presence of an SCN5A mutation. The PQ and QRS interval after Na\textsuperscript{+} blocking drugs are less accurate predictors for the presence or absence of an SCN5A mutation (parameters with AUC = 0.716 [0.543 to 0.889] \(p < 0.01\) and 0.733 [0.549 to 0.916] \(p < 0.01\), respectively [not shown]). It has been noted before \textsuperscript{17} that patients with longer baseline QRS intervals developed more QRS prolongation upon flecainide exposure than patients with normal baseline QRS intervals: a difference in underlying genotype was suggested. Na-channel blocking drugs, however, do not seem to differentiate between the two groups of patients when only the magnitude of the ST-segment elevation is considered (not shown).
We were also not able to distinguish the two groups on the basis of other characteristics such as gender, age, family history, or index event; nor was the inducibility of ventricular fibrillation/ventricular tachycardia different between the two groups.

The SCN5A mutation is reported to be found in only 15% of BS patients, and other gene(s) associated with the disorder have yet to be identified. Based on the proposed mechanism, involving exacerbation of transmural heterogeneity in action potential duration, both for ST-segment elevation and for the genesis of arrhythmias, likely candidate genes for the disorder are those encoding components (pore-forming or ancillary) of ion channels active around phase 1 of the action potential. In particular, this applies to genes displaying a transmural gradient in expression. Potential candidates include the modulatory subunits of the sodium channel, the pore-forming and modulatory components of the transient outward current, and the L-type calcium current. However, the disorder may be more complex. The region on chromosome 3 (3p22-25) linked to BS appears to contain none of these candidates, nor ion channel components that could represent homologues of these proteins. Furthermore, our study indicates that BS patients without an SCN5A mutation are expected to have normal or near-normal cardiac conduction. This suggests that the as-yet-unidentified causative gene(s) in these patients (most probably a heterogeneous group) have no impact (or a smaller impact) on conduction.

**Figure 1.** (A) PQ interval (ms) on baseline ECG for individual patients. Patients with a pore-forming region (alpha-subunit) of the human cardiac sodium channel (SCN5A) mutation were found to have a significantly longer PQ interval than patients without an SCN5A mutation. **Solid circle** = Brugada syndrome patients with known SCN5A mutation; **open circle** = mean ± SEM; **open square** = Brugada syndrome patients without known SCN5A mutation; **solid square** = mean ± SEM. (B) His to ventricle interval (HV) time (ms) for individual patients. Patients with an SCN5A mutation were found to have a significantly longer HV interval than patients without an SCN5A mutation. **Solid circle** = Brugada syndrome patients with known SCN5A mutation; **open circle** = mean ± SEM; **open square** = Brugada syndrome patients without known SCN5A mutation; **solid square** = mean ± SEM.
Figure 2. (A) Receiver operating characteristic curve comparing sensitivity and specificity of the baseline PQ interval and the presence of an SCN5A-mutation. Select PQ intervals are indicated. Below are positive predictive value and negative predictive value of various cut points of PQ interval. (B) Receiver operating curve comparing sensitivity and specificity of the His to ventricle interval (HV) and the presence of an SCN5A mutation. Select HV intervals are indicated. Below are positive predictive value and negative predictive value of various cut points of HV interval. AUC = area under the curve.
3.3.6 Study limitations
Although SSCP conditions for enhanced detection of mutations were used, the SSCP analysis technique is known to be less than 100% sensitive and varies between 70% and 95%. Moreover, we analyzed the coding regions of SCN5A for mutations. One cannot exclude the possibility of mutations occurring in regions of the gene other than coding regions. The functional impact has not been studied for all identified SCN5A mutations; therefore, a causal relationship in individual patients has not been proved.

3.2.6 Conclusions
In this study we show that the presence of conduction defects, as evidenced by a prolonged HV interval and PQ interval at baseline, and excessive QRS interval prolongation after Na+-channel blockade are more likely to be found in BS patients who are carriers of an SCN5A mutation. This represents the first step toward the identification of genotype-specific subtypes in BS that, although other responsible gene(s) have yet to be identified, will eventually facilitate genetic screening and earlier initiation of optimal therapeutic strategies.

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