Value of Electrocardiographic Parameters and Ajmaline Test in the Diagnosis of Brugada Syndrome Caused by SCN5A Mutations

Kui Hong, MD, PhD; Josep Brugada, MD, PhD; Antonio Oliva, MD; Antonio Berruezo-Sanchez, MD; Domenico Potenza, MD; Guido D. Pollevick, PhD; Alejandra Guerchicoff, PhD; Kiyotaka Matsuo, MD, PhD; Elena Burashnikov, MS; Robert Dumaine, PhD; Jeffrey A. Towbin, MD; Vladislav Nesterenko, PhD; Pedro Brugada, MD, PhD; Charles Antzelevitch, PhD; Ramon Brugada, MD

Background—The Brugada syndrome is an arrhythmogenic disease caused in part by mutations in the cardiac sodium channel gene, SCN5A. The electrocardiographic pattern characteristic of the syndrome is dynamic and is often absent in affected individuals. Sodium channel blockers are effective in unmasking carriers of the disease. However, the value of the test remains controversial.

Methods and Results—We studied 147 individuals representing 4 large families with SCN5A mutations. Of these, 104 were determined to be at possible risk for Brugada syndrome and underwent both electrocardiographic and genetic evaluation. Twenty-four individuals displayed an ECG diagnostic of Brugada syndrome at baseline. Of the remaining, 71 received intravenous ajmaline. Of the 35 genetic carriers who received ajmaline, 28 had a positive test and 7 a negative ajmaline test. The sensitivity, specificity, and positive and negative predictive values of the drug challenge were 80% (28:35), 94.4% (34:36), 93.3% (28:30), and 82.9% (34:41), respectively. Penetrance of the disease phenotype increased from 32.7% to 78.6% with the use of sodium channel blockers. In the absence of ST-segment elevation under baseline conditions, a prolonged P-R interval, but not incomplete right bundle-branch block or early repolarization patterns, indicates a high probability of an SCN5A mutation carrier.

Conclusions—In families with Brugada syndrome, the data suggest that ajmaline testing is valuable in the diagnosis of SCN5A carriers. In the absence of ST-segment elevation at baseline, family members with first-degree atrioventricular block should be suspected of carrying the mutation. An ajmaline test is often the key to making the proper diagnosis in these patients. (Circulation. 2004;110:3023-3027.)

Key Words: genetics ■ ajmaline ■ Brugada syndrome ■ sodium channel blockers

The Brugada syndrome is an arrhythmogenic disease characterized by the occurrence of sudden death in young individuals with a characteristic electrocardiographic pattern of ST-segment elevation in leads V1 to V3. Sudden cardiac death is most commonly secondary to the development of polymorphic ventricular tachycardia and fibrillation. The disease displays an autosomal dominant pattern of transmission. To date, one gene has been identified. Mutations in SCN5A, the α subunit of the sodium channel, account for ~20% of familial cases of the disease. Causative mutations reduce the availability of sodium channel current. Spontaneous fluctuation of the ECG, to the point of normalization of the ST pattern, makes identification of individuals at risk for sudden death difficult. The electrocardiographic pattern of ST-segment elevation in V1 to V3 can be unmasked in patients with a normal ECG with the use of potent sodium channel blockers. These tests, performed under close monitoring because of the possible inducibility of malignant arrhythmias, have become standard procedure in the differential diagnosis of malignant arrhythmias in patients with a structurally normal heart. This year, Rolf et al1 published a comprehensive assessment of the usefulness of ajmaline test in a large patient population, assessing the risks, diagnostic impact, and protocol.

The lower sensitivity of some of the sodium channel blockers, the variable ECG, and the lack of a “gold standard” in the diagnosis of Brugada syndrome continue to burden our ability to properly risk-stratify some of the individuals. It is

Received February 2, 2004; revision received April 15, 2004; accepted April 30, 2004.

From Masonic Medical Research Laboratory, Utica, NY (K.H., A.O., G.D.P., A.G., K.M., E.B., R.D., V.N., C.A., R.B.); Arrhythmia Unit, Cardiovascular Institute, University of Barcelona, Spain (J.B.); Cardiology Department, Hospital Universitario Puerta del Mar, Cadiz, Spain (A.B.-S.); Cardiology Department, IRCCS Casa Sollievo della Soferenza, St Giovanni Rotondo, Italy (D.P.); Pediatrics Department, Section of Cardiology, Baylor College of Medicine, Houston, Tex (J.A.T.); and Cardiovascular Research and Teaching Institute of Aalst, Belgium (P.B.).

Dr Hein J.J. Wellens was Guest Editor for this article.

Correspondence to Ramon Brugada, MD, FACC, Masonic Medical Research Laboratory, 2150 Bleecker St, Utica, NY 13501. E-mail: brugada@mmrl.edu

© 2004 American Heart Association, Inc.

Circulation is available at http://www.circulationaha.org DOI: 10.1161/01.CIR.0000144299.17008.07

3023
Figure 1. Pedigrees of the 4 families with SCN5A mutations. Squares represent males; circles, females.
not clear whether identification of the genetic defect is the best gold standard in this disease, but genetic analysis is emerging as an accurate test, if we keep in mind limitations like penetrance, expression, gender, and autonomic factors, which will play a role in delineating the final phenotype of the individual and the risk of arrhythmia. In a family with Brugada syndrome, those who are not carriers of the familial mutation (except in those rare instances where there are 2 mutations in the family) can be considered spared of the familial disease. Likewise, those who are carriers of an ion channel mutation in SCN5A are potentially at higher risk for the development of malignant arrhythmias. Genetic testing in Brugada syndrome permits accurate assessment of familial penetrance of the disease and of the value of the ajmaline test and baseline electrocardiographic parameters in identifying genetic carriers in a family. Our principal objective in the present study is to contrast the results of an ajmaline challenge and genetic screening in 4 large families with a known SCN5A mutation so as to assess the sensitivity and specificity of the pharmacological test. Our secondary aim is to assess the value of other electrocardiographic parameters in the identification of possible genetic carriers.

**Clinical Analysis**

We characterized 4 large European families comprising 147 members (Figure 1). The ECG was considered positive if the ST segment was elevated by ≥0.2 mV in at least one of the precordial leads before or after ajmaline (Figure 2).4 First-degree atrioventricular (AV) block was defined as a P-R interval longer than 200 ms. TyTe was defined as the time in milliseconds from the peak of the T wave to the end of the T wave. Right bundle-branch block (RBBB) was defined as the presence of a prolonged QRS complex (≥120 ms); rsr', rs'R', or rsR' pattern in V1 and/or V2, and wide and deep S waves in the left precordial leads; and a normal R peak time in leads V4 and V6, but ≥50 ms in lead V1.5 Incomplete RBBB was defined as the presence of rsR' pattern in V1 and/or V2 and QRS complex <120 ms. One hundred and sixteen members were relatives at possible risk of Brugada syndrome, of whom 8 were deceased with no information available (Table 1). Each of them underwent a complete physical examination and a 12-lead ECG. Twenty-four had a positive ECG at baseline. Of the remaining 84 subjects with a negative ECG at baseline, 71 (29 males and 42 females) received intravenous class I blocker (ajmaline 1 mg/kg over 5 minutes).

**Methods**

**Clinical Analysis**

We characterized 4 large European families comprising 147 members (Figure 1). The ECG was considered positive if the ST segment was elevated by ≥0.2 mV in at least one of the precordial leads before or after ajmaline (Figure 2).4 First-degree atrioventricular (AV) block was defined as a P-R interval longer than 200 ms. TyTe was defined as the time in milliseconds from the peak of the T wave to the end of the T wave. Right bundle-branch block (RBBB) was defined as the presence of a prolonged QRS complex (≥120 ms); rsr', rs'R', or rsR' pattern in V1 and/or V2, and wide and deep S waves in the left precordial leads; and a normal R peak time in leads V4 and V6, but ≥50 ms in lead V1.5 Incomplete RBBB was defined as the presence of rsR' pattern in V1 and/or V2 and QRS complex <120 ms. One hundred and sixteen members were relatives at possible risk of Brugada syndrome, of whom 8 were deceased with no information available (Table 1). Each of them underwent a complete physical examination and a 12-lead ECG. Twenty-four had a positive ECG at baseline. Of the remaining 84 subjects with a negative ECG at baseline, 71 (29 males and 42 females) received intravenous class I blocker (ajmaline 1 mg/kg over 5 minutes).

**Genetic Analysis**

The Regional Institutional Review Board approved the study, and study participants gave written consent. Blood samples (10 mL) were obtained from participating family members and spouses. Genomic DNA was isolated from peripheral blood leukocytes with the use of a commercial kit (Gentra System, Puregene). The exons of SCN5A were amplified and analyzed by direct sequencing. Polymerase chain reaction products were purified with Exosap (USB) and were directly sequenced from both directions with the ABI PRISM BigDye Terminator Cycle Sequencing Reaction and the ABI PRISM 3100-Avant Automatic DNA Sequencer.

**Statistical Analysis**

Electrocardiographic data were analyzed by unpaired t test, and a value of P<0.05 was accepted as significant. The significance of the incidence of first-degree AV block, complete RBBB, and incomplete

**TABLE 1. Genetic Data and Response to Ajmaline in the 4 Families**

<table>
<thead>
<tr>
<th>Family</th>
<th>Family members</th>
<th>Spouses</th>
<th>Members at risk</th>
<th>No clinical or genetic information</th>
<th>Genotype N/A, ECG +</th>
<th>Genotype +, ECG +</th>
<th>Genotype +, AJM +</th>
<th>Genotype +, AJM –</th>
<th>Genotype +, AJM N/A</th>
<th>Genotype –</th>
<th>Genotype –, AJM +</th>
<th>Genotype –, AJM –</th>
<th>Genotype –, AJM N/A</th>
<th>Genotype –, AJM N/A</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-011</td>
<td>38</td>
<td>8</td>
<td>30</td>
<td>4</td>
<td>3</td>
<td>11</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>12</td>
<td>8</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>IV27S+5sINSGG</td>
</tr>
<tr>
<td>24-228</td>
<td>40</td>
<td>8</td>
<td>32</td>
<td>4</td>
<td>1</td>
<td>18</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>11</td>
<td>6</td>
<td>2</td>
<td>10</td>
<td>2</td>
<td>R367H</td>
</tr>
<tr>
<td>24-064</td>
<td>50</td>
<td>12</td>
<td>38</td>
<td>2</td>
<td>1</td>
<td>22</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>12</td>
<td>13</td>
<td>7</td>
<td>11</td>
<td>1</td>
<td>R769C</td>
</tr>
<tr>
<td>24-104</td>
<td>19</td>
<td>3</td>
<td>16</td>
<td>0</td>
<td>1</td>
<td>8</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>T1620 M</td>
</tr>
</tbody>
</table>

AJM indicates ajmaline test; ECG +, individual with a positive electrocardiographic pattern at baseline.
the disease phenotype increased from 32.7% to 78.6% with patients with a negative test had the mutation. Penetrance of positive ajmaline test did not have the mutation, and 7 ajmaline tests had the mutation. Therefore, 2 patients with eight patients with positive and 7 patients with negative and 41 did not (negative ajmaline test) (Table 2). Twenty-

RBBB between carriers without ST elevation and noncarriers was calculated by the Pearson χ² test. The significance of the difference in P-R, QTc, ST elevation, and Tp-Te between the same groups of patients was calculated by ANOVA with Scheffe test for post hoc analysis. Data are presented as mean±SD.

### Results

#### Genetic Data

All 4 families had mutations in SCN5A. Genotype was positive for the SCN5A mutations in 59 of 104 individuals (25 males, 34 females) (Table 1). All patients with positive basal ECG and genetic testing had the mutation.

#### Electrocardiographic Parameters

Electrocardiographic data are shown in Table 2. There were 5 patients with RBBB (3 with ST-segment elevation and 2 without) who were carriers of the genetic mutation. None of the noncarriers had a complete RBBB. Individuals with a sodium channel mutation had a longer P-R interval than that of individuals without the mutation. First-degree AV block (defined as a P-R interval >200 ms) was more frequent in patients with the mutation (8 of 41 versus 2 of 43) (P≤0.05). QT, Tp-Te, and the presence of incomplete RBBB were no different in carriers than in noncarriers.

#### Ajmaline test

Of the 71 individuals who received the ajmaline test, 30 developed the typical ECG pattern (positive ajmaline test) and 41 did not (negative ajmaline test) (Table 2). Twenty-eight patients with positive and 7 patients with negative ajmaline tests had the mutation. Therefore, 2 patients with positive ajmaline test did not have the mutation, and 7 patients with a negative test had the mutation. Penetrance of the disease phenotype increased from 32.7% to 78.6% with the use of sodium channel blockers. The sensitivity, specificity, and positive and negative predictive values of the ajmaline test were 80%, 94.4%, 93.3%, and 82.9% respectively.

### Discussion

This article provides a comprehensive assessment of the use of the ajmaline test and electrocardiographic parameters other than ST-segment elevation to identify family member carriers of a mutation in SCN5A.

We showed in 2000⁴ that the appearance of ST-segment elevation in the right precordial leads during infusion of intravenous sodium channel blocker correlated well with the presence of an SCN5A mutation. The power of this test to uncover patients at risk for sudden death has been a matter of controversy. Recent publications have suggested that sodium channel blockers may not be as useful in making the diagnosis as previously claimed⁷; such doubts led to the search for electrocardiographic parameters that may be helpful in identifying concealed carriers. Consistent with the observations that some patients with SCN5A-mediated Brugada syndrome have conduction impairment and that there is a link between sodium channel mutations and progressive conduction disease, Smits et al⁸ showed a prolonged P-R interval in Brugada syndrome patients with SCN5A mutations when compared with those without an identified mutation. Although this might suggest that family members with no ST-segment elevation displaying conduction disease may be carriers of the genetic mutation, the study did not address this issue because it only assessed individuals affected with the disease and not their family members. Despite the absence of scientific evidence, when faced with a family with Brugada syndrome, some physicians have taken minimal conduction alterations and incomplete RBBB—very common electrocardiographic patterns in the population—as possible indicators of a mutation carrier, with important implications in these usually young individuals.
Our findings support the contention that the electrocardiographic signature of the syndrome is dynamic and often concealed and that it can be unmasked by potent sodium channel blockers such as ajmaline. We indicate in this article that the sensitivity of the ajmaline test in carriers of SCN5A mutations is 80%, and the specificity 94.4%. Two individuals are positive despite the lack of the familial mutation. Whether they are false-positive or carriers of a second mutation in another gene is unknown. The use of the ajmaline test increases the penetrance of the disease phenotype from 32% to 78%, which confirms the usefulness of the test in identifying carriers.

In a family with Brugada syndrome, the ECG at baseline is of limited value in the absence of ST-segment elevation. Clinicians should make a positive diagnosis only when the ECG presents a “coved-type” ST-segment elevation. In our data, close to 40% of patients with SCN5A mutations present with this pattern at baseline. Clinicians should suspect family members of being carriers if they show first-degree AV block and/or saddle-back ST-segment elevation without structural heart disease. A saddle-back pattern may be present in 19% of patients with SCN5A mutations who do not display a coved-type ECG. As shown in Figure 3, however, these abnormalities may be present in the general population and may confound the diagnosis. For this reason, a positive diagnosis for Brugada syndrome should never be based solely on the presence of a saddle-back pattern, and further testing is recommended. Likewise, although RBBB was only present in carriers in our study, the data did not reach statistical significance. These patients also require further workup. Finally, physicians should not draw any conclusions as to whether an individual is a carrier on the basis of the presence of incomplete RBBB or minimal alterations in conduction.

Limitations
Studies have been performed comparing the use of ajmaline and other class I sodium blockers. These studies indicate that ajmaline is the most potent agent to unmask the pattern. It is therefore possible that other sodium channel blockers may yield a lower sensitivity.

Our data assess the use of a sodium block test in a selected population of SCN5A carriers. Because the gold standard used is the genetic data, it is not possible at this point to know whether these data will hold in families with other genetic defects.

Acknowledgments
This work was supported by the Ramon Brugada Sr. Foundation; Mapfre Medicine Foundation; Institut d’Investigacions Biomédiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain; Cardiovascular Research and Teaching Institute of Aalst (CRTIA), Aalst, Belgium; the American Heart Association (National and New York State Affiliate); the Doris Duke Charitable Foundation; and the National Heart, Lung and Blood Institute of the National Institutes of Health (grants HL 47678 and HL 66169).

References