A Single Na\textsuperscript{+} Channel Mutation Causing Both Long-QT and Brugada Syndromes

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Abstract—Mutations in SCN5A, the gene encoding the cardiac Na\textsuperscript{+} channel, have been identified in 2 distinct diseases associated with sudden death: one form of the long-QT syndrome (LQT\textsubscript{3}) and the Brugada syndrome. We have screened SCN5A in a large 8-generation kindred characterized by a high incidence of nocturnal sudden death, and QT-interval prolongation and the “Brugada ECG” occurring in the same subjects. An insertion of 3 nucleotides (TGA) at position 5537, predicted to cause an insertion of aspartic acid (1795insD) in the C-terminal domain of the protein, was linked to the phenotype and was identified in all electrocardiographically affected family members. ECGs were obtained from 79 adults with a defined genetic status (carriers, n=43; noncarriers, n=36). In affected individuals, PR and QRS durations and QT intervals are prolonged (P<0.0001 for all parameters). ST segment elevation in the right precordial leads is present as well (P<0.0001). Twenty-five family members died suddenly, 16 of them during the night. Expression of wild-type and mutant Na\textsuperscript{+} channels in Xenopus oocytes revealed that the 1795insD mutation gives rise to a 7.3-mV negative shift of the steady-state inactivation curve and an 8.1-mV positive shift of the steady-state activation curve. The functional consequence of both shifts is likely to be a reduced Na\textsuperscript{+} current during the upstroke of the action potential. LQT\textsubscript{3} and Brugada syndrome are allelic disorders but may also share a common genotype. (Circ Res. 1999;85:1206-1213.)

Key Words: long-QT syndrome ▪ Brugada syndrome ▪ SCN5A ▪ arrhythmia ▪ Na\textsuperscript{+} channel

SCN5A, the gene that encodes the human cardiac Na\textsuperscript{+} channel α subunit,\textsuperscript{1} is mutated in one form of the long-QT syndrome (LQT\textsubscript{3}) and in Brugada syndrome.\textsuperscript{2,3} There are characteristic and readily distinguishable ECG patterns in these 2 syndromes. In LQT\textsubscript{3} patients, a long isoelectric ST segment precedes a peaked T wave.\textsuperscript{4} Brugada syndrome is diagnosed on the basis of characteristic ECG features in the absence of structural heart disease; these features include right precordial ST-segment elevation, which may be intermittent, and which is exacerbated by Na\textsuperscript{+} channel block and ameliorated by isoproterenol.\textsuperscript{5,6} QT intervals have been reported to be normal in patients with Brugada syndrome.\textsuperscript{5} Clinically, there appears to be some overlap between the 2 syndromes, as both exhibit a relatively high incidence of nocturnal sudden cardiac death without prior symptoms.\textsuperscript{6,8}

The prolonged QT interval in LQT\textsubscript{3}, results from persistent inward Na\textsuperscript{+} current during the plateau phase of the action potential, secondary to incomplete inactivation of mutated channels.\textsuperscript{9} Changes in the α and β subunit interaction have also been implicated.\textsuperscript{10} Although functional abnormalities have been described for Brugada syndrome–related SCN5A mutant channels,\textsuperscript{3,11} the mechanism(s) whereby these explain the Brugada phenotype are less clear.

In this study we present clinical and genetic data of a single large SCN5A-linked family, phenotypically characterized by nocturnal death and electrocardiographically by both LQT\textsubscript{3} and Brugada syndrome features in the same patients. We show that LQT\textsubscript{3} and the Brugada syndrome are more closely related than heretofore appreciated and can even be caused by the same mutation. We also report on the functional consequences of the Na\textsuperscript{+} channel mutation involved, as revealed by measuring Na\textsuperscript{+} channel activity in the Xenopus oocyte expression system.

Materials and Methods

Patients

The study was performed according to a protocol approved by the local ethics committees. Informed consent was obtained from all

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patients, who were all screened by one of us. For purposes of linkage analysis, the phenotype was assigned in a subset of the family according to the criteria described in the online Materials and Methods (see http://www.circresaha.org).

After the mutation was identified (see below), a careful history, an ECG at rest and in the supine position, and peripheral blood samples for genotype analysis were taken from as many other family members as possible. Patients with specific reasons for prolongation of the QT interval were excluded from further (electrocardiographic) analysis. We analyzed 12-lead ECGs (paper speed, 25 mm/s), only of patients ≥16 years of age, with particular reference to rate and to PR, QRS, and QT interval (the longest interval in any lead was taken). QTc was calculated according to the Bazett formula. In the final analysis, mutation carriers either are obligate carriers (by pedigree analysis) or have a proven genetic status (see below). Individuals within this family from which no DNA or ECGs were available were defined as mutation carriers when they died suddenly and unexpectedly under typical circumstances (see below).

Signal-averaged electrocardiography (SAECG) was performed in the supine position according to the method previously described for genotype analysis or have a proven genetic status (see below). Individuals in this family from which no DNA or ECGs were available were defined as mutation carriers when they died suddenly and unexpectedly under typical circumstances (see below). Individuals in this family from which no DNA or ECGs were available were defined as mutation carriers when they died suddenly and unexpectedly under typical circumstances (see below).

Statistical Analysis
Differences between groups (mutation carriers and noncarriers) were compared by the Fischer exact test or unpaired Student t test, where appropriate. A 2-tailed probability value of <0.05 was considered statistically significant. In electrophysiological studies, differences between WT Na+ current and mutant Na+ current were compared using the unpaired Student t test.

An expanded Materials and Methods section is available online at http://www.circresaha.org.

Results
The anonymized pedigree of the family is presented in Figure 1. Linkage analysis in a subset of the family (11 affected and 12 unaffected) revealed linkage to SCN5A (Table 1), whereas no evidence of linkage was detected to the LQT1, HERG, KCNQ1, and KCNE1 loci. Subsequent SSCP analysis of the coding region of SCN5A using primers flanking the exon-intron boundaries identified an aberrant conformer in exon 28 in affected family members (Figure 2; n=53, including children). The aberrant conformer was not present in unaffected family members nor in 100 alleles from unrelated control individuals. DNA sequencing of exon 28 of affected family members revealed heterozygosity for a TGA insertion at position 5537. This insertion results in the insertion of
aspartate after tyrosine 1795 (1795insD) within the highly negatively charged region of the C-terminal domain of the protein. SSCP analysis of all the other exons and direct sequence analysis of a large part of the coding region (see Materials and Methods) in 3 affected individuals (VI-27, VI-29, and VI-30) revealed no further abnormalities.

We were able to trace the history of 203 family members in 8 generations (Figure 1). ECGs were obtained from 119 individuals, of whom 79 adults had a defined genetic status (mutation carriers, n = 43; noncarriers, n = 36). Figure 3 demonstrates an example (patient VI-27). Heart rate is relatively slow, PR and QRS durations are slightly prolonged, and the QT interval is markedly prolonged (Figure 3B). In the right precordial leads, ST-segment elevation is apparent (Figure 3A). Table 2 summarizes basic demographic and electrocardiographic data of the 79 genotyped family members. Whereas sex and age are similar in affected and nonaffected members, mean heart rate is slightly lower ($P < 0.02$), and conduction parameters (PR and QRS intervals) are slightly prolonged in mutation carriers (for both parameters; $P < 0.0001$). In addition, HV interval was prolonged in 4 of the 5 carriers in whom an invasive electrophysiological study was performed: 58, 78, 75, and 80 ms in V-1, VI-27, VI-54, and VI-60 respectively, and 50 in VI-3. SAECG was abnormal in 23 of 29 mutation carriers tested (79%) and abnormal in 2 of 14 noncarriers (14%; $P < 0.001$). Figure 4 depicts normalized QT intervals (QTc) versus heart rate in analyzed patients. QTc was clearly prolonged in the vast majority of mutation carriers, in particular in those in whom heart rate is slow. PR and QRS prolongation was concomitantly present in 14 carriers, whereas only PR or only QRS prolongation was seen in 10 carriers (and in 6 noncarriers) and 10 carriers (and in 1 noncarrier), respectively (Figure 5A). ST-segment elevation was present in 21 of the 43 carriers versus 3 of 36 noncarriers ($P < 0.001$; mean values in Table 2). Figure 5B shows QTc intervals versus ST segment elevation in individuals. In 16 carriers, both QTc is prolonged and right precordial ST segments are elevated. In 13 carriers, only QTc was prolonged, whereas in 5 carriers (and 3 noncarriers) only ST-segment elevation was apparent. In all 3 carriers (VI-27, VI-29, and VI-30) who received a bolus procainamide (250 mg IV), ST-segment elevation was increased further (see inset, Figure 3B). There were no echocardiographic abnormalities in 29 mutation carriers.

Unexpected nocturnal sudden cardiac death was the only symptom in this family, occurring in 16 family members since 1905 (10 female, 6 male; see online Table, available at http://www.circresaha.org). Eight patients died suddenly under unknown circumstances. One died in the chair of the barber while being shaved (IV-8). Death was witnessed in 5 cases, occurring between 4:00 and 7:00 AM, and the episodes were characterized by sudden onset of gurgling and gasping,

### Table 1. Linkage Analysis of Chromosome 3p21 Markers and the Affected Status

<table>
<thead>
<tr>
<th>Marker</th>
<th>Lod score at $\theta$</th>
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<tbody>
<tr>
<td>D3S1211</td>
<td>$-\infty$ 2.26 1.72 1.01 0.32</td>
</tr>
<tr>
<td>SCN16</td>
<td>3.68 2.82 2.00 1.20 0.52</td>
</tr>
<tr>
<td>SCN5A 1795insD</td>
<td>6.50 5.41 4.20 2.85 1.35</td>
</tr>
<tr>
<td>D3S1298</td>
<td>2.60 2.26 1.78 1.19 0.54</td>
</tr>
<tr>
<td>D3S1100</td>
<td>5.78 5.48 4.38 2.16 0.85</td>
</tr>
</tbody>
</table>

Figure 2. Insertion of 3 nucleotides (TGA) at position 5537, resulting in the addition of aspartic acid in the SCN5A protein of affected individuals. A, Representative part of the pedigree (for symbols see Figure 1). B, SSCP analysis of exon 28 of SCN5A, with a distinct shift (arrow) in mutation carriers. C, Sequence analysis of exon 28 of SCN5A in mutation carriers and non–mutation carriers, showing the insertion of TGA resulting in the addition of aspartic acid after the tyrosine at position 1795 of the SCN5A protein (arrow).
and moaning respiration. Patients were unconscious and could not be awakened. No electrocardiographic recordings are available from these episodes. Previous ECGs were available in 4, all demonstrating bradycardia with significant QT-segment prolongation. Nine victims were obligate carriers of the aberrant gene. Three clinically affected individuals have been evaluated in hospital, and sudden arousal during the early morning hours did not reveal any (additional) electrocardiographic abnormality. The mean age (±SD) of sudden cardiac death victims was 32.3±14.63 (n=22), with 19 individuals ≤40 years (male/female ratio, 9/10).

To establish the consequences of the 1795insD insertion on the electrophysiological properties of the Na⁺ channel, macroscopic Na⁺ currents (I\(_{Na}\)) were recorded in oocytes injected with cRNA encoding either the WT or the 1795insD mutant Na⁺ channel α subunit. Figure 6A shows typical families of
Na⁺ current traces elicited by 5-mV depolarizing steps between −90 and +40 mV from a holding potential of −100 mV. There was a striking difference in peak amplitudes between the WT Na⁺ current (I_{Na,WT}, Figure 6A, left) and the 1795insD mutant Na⁺ current (I_{Na,1795}, Figure 6A, right), despite the fact that similar amounts of cRNA were injected. The average (±SEM) current-voltage relations in Figure 6B show that the maximal I_{Na} amplitude was 9.9±1.7 μA (n=14) and 2.2±0.5 (n=22) for I_{Na,WT} and I_{Na,1795} respectively. The averaged data were obtained from 6 different batches of oocytes. The much larger peak amplitude of WT Na⁺ currents compared with 1795insD Na⁺ currents was a consistent finding. In addition, the voltage for both the threshold of activation and the maximum peak current was shifted by +5 mV for 1795insD channels. We also determined the steady-state voltage dependence of activation and inactivation for I_{Na,WT} and I_{Na,1795}, as illustrated in Figure 6C. The averaged data points of the inactivation curve were fitted with a Boltzmann function with V_{1/2} of −78.7 mV and a k of −4.5 for the WT Na⁺ channel (n=21) and a V_{1/2} of −86.0 mV and a k of −5.0 for the 1795insD mutant Na⁺ channel (n=22). These results indicate a negative shift of the inactivation curve of the 1795insD mutant by 7.3 mV. The averaged data points of the activation curve were fitted with a Boltzmann function with a V_{1/2} of −40.2 mV and a k of 5.3 for the WT Na⁺ channel (n=20) and a V_{1/2} of −32.1 mV and a k of 5.7 for the 1795insD mutant Na⁺ channel (n=22), resulting in an 8.1-mV positive shift of the activation curve of the 1795insD mutant. Both shifts will result in a reduced Na⁺ current during the upstroke of the action potential and a reduced Na⁺ window current. Recovery from inactivation (Figure 6D) was slightly, but significantly, slower for the 1795insD mutant channel. When the data were fitted with a single exponential function, mean time constants (±SEM) were 12.2±0.6 ms (n=22) and 14.7±0.7 ms (n=22) for the WT and the 1795insD mutant Na⁺ channel, respectively.

Because LQT3 has been associated with incomplete inactivation of the Na⁺ channel, resulting in a persistent Na⁺ current, we sought to determine whether a reduced rate of inactivation or incomplete inactivation was also present in our 1795insD mutant Na⁺ channel. I_{Na,WT} and I_{Na,1795} were recorded at −20 mV, and current decay was fitted with either a single- or double-exponential function (not shown). The results showed that both the fast and the slow time constant of inactivation were only slightly, and not significantly, larger for the 1795insD Na⁺ channel (mean±SEM, WT: τ_{fast}=0.98±0.06, τ_{slow}=7.1±0.7 [n=20]; 1795insD: τ_{fast}=1.15±0.06, τ_{slow}=10.49±2.9 [n=22]). Also, the study of procainamide- and tetrodotoxin-sensitive 1795insD Na⁺ currents did not reveal the presence of a persistent inward current. Because 1795insD Na⁺ currents were of very small amplitude, we considered the possibility that a persistent inward current, usually <2% of the peak current, was too small to distinguish. Unfortunately, attempts to increase the expression level by injecting 5 to 10 times higher amounts of cRNA increased peak 1795insD Na⁺ currents no further than 3 μA.

**Table 2. Patient Basic Demographic and ECG Characteristics (mean±SD)**

<table>
<thead>
<tr>
<th>Significance, P&lt;</th>
<th>Number</th>
<th>Male/Female, %</th>
<th>Age, y</th>
<th>Rate, bpm</th>
<th>QTc, ms</th>
<th>PQ, ms</th>
<th>QRS, ms</th>
<th>STelev, mm</th>
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</thead>
<tbody>
<tr>
<td>Carriers</td>
<td>43</td>
<td>47/53</td>
<td>41.0±14.31</td>
<td>67.5±16.41</td>
<td>489.4±44.09</td>
<td>201.0±19.71</td>
<td>117.6±14.36</td>
<td>1.58±1.04</td>
</tr>
<tr>
<td>Noncarriers</td>
<td>36</td>
<td>47/53</td>
<td>39.4±18.37</td>
<td>74.5±13.50</td>
<td>403.7±24.82</td>
<td>156.9±31.71</td>
<td>93.4±13.25</td>
<td>0.36±0.56</td>
</tr>
</tbody>
</table>

PR and QRS are the PR interval, measured from the onset of the P-wave to the onset of the QRS complex, and the duration of the QRS complex, respectively. For measurement of QTc and ST segment elevation (STelev), see Materials and Methods.

**Figure 4.** QTc vs RR interval in all individuals analyzed (43 mutation carriers [■] and 36 noncarriers [□]). QT intervals are particularly prolonged at long RR intervals. However, also at normal heart rate (RR 600 to 1000 ms), QT interval is prolonged in most carriers.

**Figure 5.** PR vs QRS interval (A) and ST-segment elevation vs QTc (B). A, Upper limit of normal values is indicated by dotted lines. B, Values indicated by dotted lines are those used for the linkage study. Note that exclusively mutation carriers have combined PR and QRS interval prolongation (A) and combined ST-segment elevation and QTc prolongation (B). See text for further discussion.
Discussion

We describe a single, large SCN5A-linked family with phenotypic characteristics of both LQT3 and Brugada syndrome in the same patients. QT-interval prolongation and abnormal T-wave configuration are seen particularly at slow heart rates, and as shown previously in individual patients in this family, normalization occurs as rate increases.12 Similar steep APD- and QT-rate relationships have been observed in LQT3 patients8 and in experimental models mimicking LQT3.17 It is likely that normal K+ currents result in physiological or supraphysiological cardiac repolarization during fast rates in these patients. In contrast, incomplete inactivation of INa (as a result of mutation in the SCN5A gene) results in abnormal repolarization at slow rates.7,9 Further compounding the QT abnormality is the bradycardia seen in affected patients (Figure 4), as has been reported for other LQT3 patients.10 It is likely that these bradycardia-induced QT-interval abnormalities and resultant torsade de pointes underlie the high incidence of nocturnal sudden death in this and other LQT families.7,8 Alternatively, a high vagal tone, likely of importance at night. This is especially the case in the syndrome of sudden unexplained death in young South-Asian men, which appears to be part of the clinical spectrum.20 Causally related enhancement of vagal tone, just before the (fatal) arrhythmia, has been suggested,18 and acetylcholine augments the ST-segment changes.21 Note that 1 of our patients died suddenly while being shaved (potentially a carotis sinus massage, which may increase vagal tone). In the family, significant ST-segment elevation was present in 49% of patients. Procainamide exaggerated the effects in the 3 patients in which it was tested (Figure 3). In addition, intraventricular conduction defects (prolonged QRS and HV interval, late potentials) were generally present. Although the conduction delay is mild, the concomitant presence of both PR and QRS conduction delay in a significant subset of affected patients (Figure 5A) suggests a hampered conduction in different cardiac compartments. These features are all compatible with the Brugada syndrome diagnosis. Importantly, 16 affected subjects displayed both right precordial ST-segment elevation and QT-prolongation (Figures 3 and 5B).

In affected family members, 3 nucleotides (TGA) were inserted at nucleotide position 5537. This mutation gives rise to the insertion of a charged amino acid (Asp) after residue 1795 in the C-terminal end of the Na+ channel. Data are mean±SEM. C, Steady-state voltage dependence of activation for WT (C, n=21) and 1795insD mutant (D, n=22) Na+ channels, and of inactivation for WT (C, n=21) and 1795insD mutant (D, n=22) Na+ channels. See text for further discussion. D, Recovery from inactivation of WT (C) and 1795insD mutant (D) Na+ channels at –100 mV. Inset, Bar histogram showing average time constants for WT (n=22) and 1795insD mutant (n=22) Na+ channels. See text for further discussion. *P<0.05.
action potentials are proportionately well represented and exhibit a particularly well established “spike and dome morphology.” 

Indeed, in our and other patients with the Brugada syndrome,6,21 reduction of $I_{Na}$ by Na$^+$ channel blockers augments the ST-segment abnormalities (Figure 3). The results from our expression study are in line with a reduced Na$^+$ current. We found a 7.3-mV negative shift of the steady-state inactivation curve and an 8.1-mV positive shift of the steady-state activation curve of the 1795insD mutant channels. The functional consequence of both shifts is likely to be a reduced Na$^+$ current during the upstroke and phase 1 of the action potential. Moreover, 1795insD Na$^+$ currents had 5-fold smaller amplitudes than WT currents, which is less than expected on ground of the shifts in activation- and inactivation curves alone. It suggests the presence of additional differences, such as a reduced Na$^+$ channel density or conductance. Our findings are different from functional characterization of the Brugada syndrome SCN5A mutations described so far. These included faster recovery from inactivation and a negative shift of the steady-state activation curve.3,11

It is difficult to link the prolonged QT interval in these patients to the observed kinetic characteristics of the 1795insD mutant channel. In general, prolongation of the repolarization process suggests an increase in net inward current during the plateau phase of the action potential. Indeed, it has been shown that persistent inward Na$^+$ current, secondary to incomplete inactivation, underlies LQT$_3$.9 Analysis of procarbamide- and tetrodotoxin-sensitive currents did not reveal such a persistent Na$^+$ current in our study. The observed small increase in inactivation time constants is probably not sufficient to account for the prolonged QT interval, certainly not in view of the overall reduction in $I_{Na}$ amplitude.

To ultimately decide on the presence (or absence) of a persistent inward current, further experiments are needed. Several factors may have hampered its detection in the present study. First, experiments were performed at room temperature. It has been shown that the kinetics of both WT and ΔKPQ Na$^+$ channels are highly sensitive to temperature, having 2-fold faster activation and inactivation kinetics at 33°C compared with 23°C and a positive shift of the voltage dependence of $I_{Na}$ inactivation curves alone. It suggests the presence of additional differences, such as a reduced Na$^+$ channel density or conductance. Our findings are different from functional characterization of the Brugada syndrome SCN5A mutations described so far. These included faster recovery from inactivation and a negative shift of the steady-state activation curve.3,11

In summary, we describe a large SCN5A-linked family, characterized by QT prolongation, in particular during bradycardia; discrete conduction disturbances throughout the heart; and nocturnal sudden cardiac death. Electrocardiographic features of LQT$_1$ and Brugada syndrome are present in the same (affected) individuals, demonstrating that LQT$_1$ and Brugada syndrome are both more closely related than heretofore appreciated. Both syndromes can even be caused by the same mutation.

Acknowledgments

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References


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