A Mutation in the β3 Subunit of the Cardiac Sodium Channel Associated With Brugada ECG Phenotype

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Background—Brugada syndrome, characterized by ST-segment elevation in the right precordial ECG leads and the development of life-threatening ventricular arrhythmias, has been associated with mutations in 6 different genes. We identify and characterize a mutation in a new gene.

Methods and Results—A 64-year-old white male displayed a type 1 ST-segment elevation in V1 and V2 during procainamide challenge. Polymerase chain reaction–based direct sequencing was performed using a candidate gene approach. A missense mutation (L10P) was detected in exon 1 of SCN3B, the β3 subunit of the cardiac sodium channel, but not in any other gene known to be associated with Brugada syndrome or in 296 controls. Wild-type (WT) and mutant genes were expressed in TSA201 cells and studied using whole-cell patch-clamp techniques. Coexpression of SCN5A/WT + SCN1B/WT + SCN3B/L10P resulted in an 82.6% decrease in peak sodium current density, accelerated inactivation, slowed reactivation, and a −9.6-mV shift of half-inactivation voltage compared with SCN5A/WT + SCN1B/WT + SCN3B/WT. Confocal microscopy revealed that SCN5A/WT channels tagged with green fluorescent protein are localized to the cell surface when coexpressed with WT SCN1B and SCN3B but remain trapped in intracellular organelles when coexpressed with SCN1B/WT and SCN3B/L10P. Western blot analysis confirmed the presence of Na+β3 in human ventricular myocardium.

Conclusions—Our results provide support for the hypothesis that mutations in SCN3B can lead to loss of transport and functional expression of the hNav1.5 protein, leading to reduction in sodium channel current and clinical manifestation of a Brugada phenotype. (Circ Cardiovasc Genet. 2009;2:270-278.)

Key Words: Brugada syndrome ▪ arrhythmia ▪ ion channels ▪ SCN3B ▪ protein trafficking

Brugada syndrome (BrS) is a cardiac channelopathy characterized by ST-segment elevation or the appearance of accentuated J waves in the right precordial leads (V1 to V3) of the ECG and the development of life-threatening polymorphic ventricular tachycardia. The ECG characteristics of the BrS are dynamic and often concealed but can be unmasked by potent sodium channel blockers.1,2

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BrS has been associated with mutations in 6 different genes. Mutations in SCN5A (Na,1.5, BrS1) have been reported in 11% to 28% of BrS probands, CACNA1C (Ca,1.2, BrS3) in 6.7%, CACNB2b (Ca,β2b, BrS4) in 4.8% and mutations in glycerol-3-phosphate dehydrogenase 1-like enzyme gene (GPD1L, BrS2), β1-subunit of sodium channel (SCN1B, BrS5), and MiRP2 (KCNE3; BrS6) are much more rare.3–8 These genetic defects lead to development of BrS secondary to either a loss of function of sodium (INa) or L-type calcium (ICa) channel current, or a gain of function of transient outward current (Ito). Thus, ≈72% of BrS probands remain genotype negative. Here, we report the identification of another gene associated with the BrS phenotype caused by loss of function of INa secondary to a mutation in the β3-subunit of the cardiac sodium channel, encoded by SCN3B.

Methods

Mutation Analysis

Genomic DNA was prepared from peripheral blood lymphocytes of the patient. Genomic DNA was extracted from peripheral blood leukocytes using a commercial kit (Genatra System, Puregene, Valencia, Calif). All known exons of the Brugada-susceptibility genes (SCN5A, IRX5, SCN1B, SCN3B, CACNA1C, KCNE2, KCNE3, and GPD1L) were amplified with intronic primers and sequenced in both directions to probe for mutations, with the use of

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an ABI PRISM 3100-Avant Automatic DNA sequencer (Applied Biosystem, Foster City, Calif). The sequence primers of SCN3B are shown in Table 1 (reference sequence, NM_018400). One hundred twenty individuals, matched by race and ethnic background, with no history of cardiac arrhythmias were used as controls.

Site-Directed Mutagenesis and Transfection of the TSA201 Cell Line

For patch-clamp study, site-directed mutagenesis was performed with QuikChange (Stratagene, La Jolla, Calif) on full-length human wild-type (WT) and mutant SCN3B cDNA cloned in pRES2-DsRed-Express (RFP) vector, the WT SCN3B cloned in pRES2-AcGFP1 vector, and the WT SCN5A cloned in pCfnDNA3.1. SCN3B was a gift from Dr Takashi Tokino (Japan). In the case of trafficking studies, human WT SCN5A cDNA cloned in pCdfDNA3.1 was fused with green fluorescent protein (GFP) (ATG) at the C-terminus for coexpression with the expression of Nav1.5 fluorescence intensity at the plasma membrane region (2 μm). Confocal microscopy was used to localize the channels and identify trafficking defects. Briefly, cells were grown on polysulphonic coated 35-mm culture dishes and studied 48 hours posttransfection. Experiments were performed on an Olympus Fluoview laser-scanning confocal microscope (Olympus, Orangeburg, NY) and images were acquired with Fluoview acquisition software program on a personal computer. GFP-labeled cells were analyzed in the x/z configuration. An argon laser was focused from 488 nm and the emission light was collected at 520 nm in photomultiplier tube 1. Transmission image was acquired in photomultiplier tube 2. Fluorescence signals were collected with either a 40× or a 60× oil-immersion objective lens. Image analysis of the z series stack was measured and the ratio of peripheral to total cell area fluorescence was calculated. Analysis of GFP-labeled cells was performed using both Fluoview and Image J software.

Western Blot Analysis

Membrane proteins (50 µg/lane, except for TSA201-SCN3B 0.5 µg/lane) were run on 5% to 15% gradient linear SDS-PAGE. Bio-RAD 161 to 0374 Precision Plus Protein Dual Color Standards 10 λ/lane was used as a molecular marker reference. Rabbit anti-human SCN3B polyclonal antibody at 1:500 (ab48552, Abcam, Cambridge, Mass) was used to detect bands in membrane proteins from untransfected TSA201 cells (negative control). SCN3B transfected TSA201 cells (positive control), and from 2 human left ventricular samples.

Statistical Analysis

Data are presented as mean±SEM. A 2-tailed Student t test was used for statistical comparison of 2 groups and ANOVA coupled with Student-Newman-Keuls test for comparison of 3 or more groups (SigmaStat, Jandel Scientific Software, San Rafael, Calif). Differences were considered statistically significant at a value of P<0.05.

Results

Index Case

The study was approved by the regional institutional review board. The proband, a 64-year old white man (German, Swedish, and Native American descent), presented with a resting ECG displaying a slight ST-segment elevation and

Table 1. Oligonucleotide Primers for Genetic Analysis of Na,β,2 Subunits

<table>
<thead>
<tr>
<th>Exon</th>
<th>Forward Primer (5 to 3)</th>
<th>Reverse Primer (5 to 3)</th>
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<tbody>
<tr>
<td>1</td>
<td>CCAAGACTTCTTTTGTGAC</td>
<td>CAGCTGGAGACACTTAGTAG</td>
</tr>
<tr>
<td>2</td>
<td>GCTGTCAGTTTACGTGAG</td>
<td>GTAAAGTGTAGCAGTGATGCC</td>
</tr>
<tr>
<td>3</td>
<td>CATCTTTTAGGGCTTCAC</td>
<td>CTTGCATGCACTATCTGC</td>
</tr>
<tr>
<td>4</td>
<td>CTTCGACATTCTTGTGATTC</td>
<td>GTATAATAGGTGCCTATCTC</td>
</tr>
<tr>
<td>5</td>
<td>GGGTAGACAGTGACCTAAG</td>
<td>CTTGTACATATATGCGGGG</td>
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INa was elicited by depolarizing pulses ranging from −90 to +30 mV in 5-mV increments with a holding potential of −120 mV. Peak currents were measured and Imax densities (pA/pF) were obtained by dividing the peak Imax by the cell capacitance obtained. Activation properties were determined from I/V relationships by normalizing peak Imax to driving force and maximal Imax, and plotting normalized conductance versus Vm. Voltage-dependence of steady-state inactivation was obtained by plotting the normalized peak current (40-ms test pulse) to −20 mV after a 1000-ms conditioning pulse from −140 to −60 mV with the holding potential of −120 mV versus Vm. The activation and steady-state inactivation curves were fitted to the Boltzmann equation.

V1/2 = Vm − (1 + exp((V − V1/2)/k))V1/2/k, to determine the membrane potential for half-maximal activation V1/2 and the slope factor k. Pulses for recovery from inactivation were of 20-ms duration. Peak current elicited during the second pulse was normalized to the value obtained during the initial test pulse. It was determined by fitting data to a double exponential function: I(t)/Imax = A1(1−exp(−τ1t))+A2(1−exp(−τ2t)), where A1 and A2 are the fractions of fast and slow inactivating components, respectively, and τ1 and τ2 are their time constants.

All data acquisition and analysis were performed using pCLAMP version 9.2 (Axon Instruments), Excel (Microsoft, Redmond, Wash), and Origin 7.5 (Microcal Software, Northampton, Mass).

Localization of Na+ Channels

We assessed channel trafficking using Na+ channels α-subunit (SCN5A) tagged with GFP. Confocal microscopy was used to localize the channels and identify trafficking defects. Briefly, cells were grown on polysulphonic coated 35-mm culture dishes and studied 48 hours posttransfection. Experiments were performed on an Olympus Fluoview laser-scanning confocal microscope (Olympus, Orangeburg, NY) and images were acquired with Fluoview acquisition software program on a personal computer. GFP-labeled cells were analyzed in the x/z configuration. An argon laser was focused from 488 nm and the emission light was collected at 520 nm in photomultiplier tube 1. Transmission image was acquired in photomultiplier tube 2. Fluorescence signals were collected with either a 40× or a 60× oil-immersion objective lens. Image analysis of the z series stack was measured and the ratio of peripheral to total cell area fluorescence was calculated. Analysis of GFP-labeled cells was performed using both Fluoview and Image J software.

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negative T wave in V1 suggestive, but not diagnostic, of BrS. A type 1 ST-segment elevation, diagnostic of a BrS, was unmasked in leads V1 and V2 with sodium channel blockade using procainamide (Figure 1). ECG characteristics are summarized in Table 2. PR interval was 180 ms at baseline and increased to 200 ms after procainamide. An internal cardioverter defibrillator was implanted in 2005. The proband was asymptomatic and did not have a family history of sudden cardiac death. Family history was remarkable for a high incidence of cancer. The proband had 2 paternal aunts and 3 paternal uncles, all of whom died of lung cancer. Interrogation of the internal cardioverter defibrillator in July of 2008 revealed an episode of atrial flutter with 2:1 atrioventricular block.

Molecular Genetics
Genetic analysis of 9 Brugada-susceptibility genes (SCN5A, SCN1B, CaCNB2B, CaVI.2, IRX5, KCNE2, KCNE3, KCNE4, and GPD1L) proved negative. Further genetic screening of the sodium channel β-subunits revealed a novel missense mutation in exon 1 of SCN3B. Polymerase chain reaction–based sequencing analysis revealed a double peak in the sequence of exon 1 of the SCN3B gene (Figure 2A) showing a T-to-C transversion at nucleotide 29 (arrow) in index case. The mutation predicts a substitution of proline (CCG) for leucine (CTG) at position 10 (L10P). B, Location of the L10P mutation in SCN3B is indicated with SCN5A and SCN1B using a conventional transmembrane topology model. C, Alignment of the voltage-gated sodium channel β3-subunit family amino acid sequence, with related sequence shows that L10 (in gray square background) is highly conserved among different mammalian species.

Electrophysiological Characteristics of SCN5A Coexpressed With WT and Mutant SCN3B in TSA201 Cells
SCN5A/WT+SCN1B/WT, SCN5A/WT+SCN1B/L10P/WT, or SCN5A/WT+SCN1B/WT+SCN3B/L10P were ex-

<table>
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<th>Table 2. ECG Characteristics of Affected Patient</th>
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<tr>
<td>RR Interval, ms</td>
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<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Baseline</td>
</tr>
<tr>
<td>After procainamide</td>
</tr>
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</table>

Figure 1. ECG of the index patient before and after sodium channel block challenge. A, 12-lead ECG at baseline. B, ECG recorded 10 minutes after procainamide infusion. ECG shows accentuation of r' and development of a type 1 ST-segment elevation in V1 and V2 (arrows).
pressed in TSA201 cells to assess the effects of the mutation on sodium channel function. Figure 3A shows macroscopic currents recorded from these channels together with the current-voltage relationships. Maximum peak inward current occurred at a potential of −35 mV for all channel types. Coexpression of SCN3B/WT with SCN5A/WT+SCN1B/WT increased peak current density from −281.3±62.3 pA/pF to −402.8±93.2 pA/pF (n=9 and 13, respectively; P<0.05 between 2 groups). Coexpression of SCN3B/L10P resulted in a marked decrease in peak sodium current density to −70.2±14.5 pA/pF (n=25; 17.4% of SCN5A/WT+SCN1B/WT+SCN3B/WT and 25.0% of SCN5A/WT+SCN1B/WT current density; P<0.05 for each; Figure 3B). Coexpression of SCN3B/L10P produced total loss of function in 40% of cells studied (10 of 25 cells, Figure 3A).

The half-inactivation voltage (V1/2) of mutant $I_{Na}$ channels (SCN5A/WT+SCN1B/WT+SCN3B/L10P) was 14.8 and 9.6 mV more negative than those of SCN5A/WT+SCN1B/WT and SCN5A/WT+SCN1B/WT+SCN3B/WT channels, respectively (P<0.01, respectively; Table 3 and Figure 4B). Steady-state activation, obtained after applying the step protocol in inset of Figure 3A, was similar among the 3 groups (Figure 4B). Recovery from inactivation, measured using a standard double-paired pulse protocol, was similar in the 2 control groups but slower in the mutant channels (P<0.01, respectively; Table 3 and Figure 4C). The L10P mutation caused a shift in the voltage dependence of steady-state inactivation and slowed recovery from inactivation, thus serving to further reduce sodium channel availability.

**Figure 3.** Effect of SCN3B mutation on sodium channel current recorded in TSA201 cells. A, Representative sodium current traces in TSA201 cells expressing SCN5A/WT+SCN1B/WT alone or cotransfected with SCN3B/WT or SCN3B/L10P. All were recorded 48 hours after transfection. Upper right and left panel shows WT of SCN5A+SCN1B and SCN5A+SCN1B+SCN3B. Two SCN3B/L10P traces are shown in the lower grouping, 1 in which there was partial loss of function (1) and another in which there was total loss of function (2). The inset shows the current-voltage relationship (CVR) protocol employed. B, Current-voltage relationship for SCN5A/WT+SCN1B/WT (n=9), SCN5A/WT+SCN1B/WT+SCN3B/WT (n=13), and SCN5A/WT+SCN1B/WT+SCN3B/L10P (n=25) current density. Peak current density was significantly reduced for SCN5A/WT+SCN1B/WT+SCN3B/L10P when compared with SCN5A/WT+SCN1B/WT and SCN5A/WT+SCN1B/WT+SCN3B/WT (values are mean±SEM; $P<0.01$ for each).

**Traffic of SCN3A Coexpressed With SCN3B/L10P Mutant in TSA201 Cells**

To evaluate whether the loss of function caused by the SCN3B/L10P mutation is due in part to a trafficking defect, we studied GFP-fusion-SCN5A/WT coexpressed with SCN1B/WT alone, or combined with either SCN3B/WT or SCN3B/L10P. $xyz$ scans of SCN5A/WT+SCN1B/WT on the confocal microscope revealed both a central and peripheral pattern of staining suggesting localization of the channel in the cell membrane and intracellular organelles (Figure 5A through 5C). Protein expression of SCN5A/WT channels was enhanced when both SCN1B/WT and SCN3B/WT were added (Figure 5D through 5F). In contrast, SCN3B/L10P resulted in internal staining consistent with intracellular compartmentalization with no evidence of plasma-member staining (Figure 5G through 5I), suggesting that channels were trapped in the endoplasmic reticulum and/or Golgi complex. The ratio of peripheral to total cell area fluorescence intensity was similar for SCN5A/WT.
WT/SCN1B/WT and SCN5A/WT/H11001 SCN1B/WT/H11001 SCN3B/WT (P/H11022 0.05), but significantly reduced for the mutant channel (SCN5A/WT/H11001 SCN1B/WT/H11001 SCN3B/L10P; P/H11021 0.01; Figure 5J).

These results demonstrate that β-subunit mutations can lead to impaired trafficking of the cardiac sodium channel.

**Na,β3 Expression in the Human Heart**

Figure 6 shows the results of Western blot analysis performed to confirm the presence of Naβ3 in human ventricular myocardium. Anti-human SCN3B antibody detected a unique band in membrane proteins from SCN3B transfected TSA201 cells (positive control) and from 2 human left ventricular myocardial preparations, but not in untransfected TSA201 cells (negative control).

**Discussion**

Voltage-gated sodium channels are vital to the function of excitable cells including those comprising the heart. Auxiliary or β-subunits are known to provide functional diversity among sodium channels. They do not form the ion-conducting pore, but are multifunctional proteins that play critical roles in modulation of channel function, regulation of channel expression levels at the plasma membrane, and cell adhesion.10 To date, 4 different Na,β subunits have been

**Table 3. Effects of β-Subunit Coexpression on Equilibrium Gating Parameters**

<table>
<thead>
<tr>
<th>Subunit</th>
<th>Inactivation</th>
<th>Activation</th>
<th>Recovery</th>
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<tbody>
<tr>
<td></td>
<td>V1/2, mV</td>
<td>K, mV</td>
<td>n</td>
</tr>
<tr>
<td>SCN5A/WT+1B/WT+3B/WT</td>
<td>-95.97±1.48</td>
<td>5.33±0.64</td>
<td>10</td>
</tr>
<tr>
<td>SCN5A/WT+1B/WT+3B/L10P</td>
<td>-105.56±1.80†</td>
<td>5.44±0.33</td>
<td>11</td>
</tr>
<tr>
<td>SCN5A/WT+1B/WT</td>
<td>-90.78±1.02†</td>
<td>5.30±0.20</td>
<td>7</td>
</tr>
</tbody>
</table>

Parameters of inactivation and activation were calculated from the Boltzmann function (see Methods section). V1/2 is the voltage for half-maximal availability or activation and k is the slope factor. Parameters of recovery were fitted to a double exponential function (see Methods). τf and τs are the time constant for the fast and slow gating modes, respectively, of recovery. Data from Figure 4B and 4C are means±SEM.

*P<0.01 versus SCN5A/WT+1B/WT+3B/WT.
†P<0.01 versus SCN5A/WT+1B/WT.
‡P<0.05 versus SCN5A/WT+1B/WT+3B/WT.

Figure 4. Functional characterization of mutant sodium channels. A, Representative traces recorded from WT and mutant channels in response to the voltage clamp protocol depicted on right middle inset. B, Steady-state voltage dependence of activation (right plot) and inactivation (left plot) of SCN5A/WT+SCN1B/WT and cotransfection of either SCN3B/WT or SCN3B/L10P. Boltzmann curves were fitted to both activation and steady-state inactivation data. Averaged values and the number of cells used are presented in Table 3. C, Recovery from fast inactivation of WT and L10P mutant channels determined. The 2-pulse protocol is shown in the inset. Fitting to a double-exponential function yielded the time constants as demonstrated in Table 3. τf and τs in the L10P group were significantly slower as compared with 2 WT groups. Data are shown as mean±SEM.
described (SCN1B, SCN2B, SCN3B, and SCN4B), and shown to play a critical role in cell adhesion, signal transduction, channel expression at the plasma membrane, and voltage dependence of channel gating. All are detectable in cardiac tissue. β1A, a splice variant of SCN1B, is expressed in embryonic brain and adult heart in rat. The distribution and expression level of sodium channel α- and β-subunits in human and canine hearts has not been well characterized.

**Sodium Channel β-Subunits Modulate Sodium Channel in Heart**

Because of their significant role in modulating channel expression and function, genes that encode cardiac channel β-subunit proteins are attractive candidates for ion channelopathies like BrS. The role of β1-subunits have been studied most extensively. β1-coexpression has been reported to have no observable effect on SCN5A function, to result in increased sodium current density with no detectable effects on channel kinetics or voltage dependence, to modulate channel sensitivity to lidocaine block with subtle changes in channel kinetics and gating properties as well as to shift the voltage dependence of steady-state inactivation or alter the rate of recovery from inactivation. Previous studies involving coexpression of SCN5A with β3 have reported (1) increased current density, a depolarizing shift in the voltage dependence of inactivation, and an increased rate of recovery from inactivation in Xenopus oocytes, or (2) a hyperpolarizing shift of inactivation, slowed recovery from inactivation, and reduced late sodium channel current, without any change in peak current density in Chinese hamster ovary-K1 cells.
The role of 

increase in late INa giving rise to the LQT3 variant of the 

is also responsible for forming the Ig fold for ß1 and ß3, a 

disruption of which can cause an inherited epilepsy syn-

tion site for 

C24, C21 and C96 are believed to correspond to the interac-

Functional and trafficking defects in cardiac sodium channel 

Although mutations in Nav1.1- and Nav1.3-subunits have been associated 

and AV block strongly suggests that sodium channel dysfunction is a major 

Limitations of the Study 

In addition, we provide evidence that SCN3B is a 

BrS-susceptibility gene. An L10P missense mutation in a 

A genotype-phenotype correlation between the 

family members died of lung cancer. SCN3B levels are upregulated in human cancer cell lines by DNA damage 

Results suggest that SCN3B mediates a p53-dependent apoptotic pathway and may be a candidate for 

An interesting aspect of the family studied was that many 

A native American cohort was not available for study as a control group. 

were available for study. A native American cohort was not 

same mutation in the ß3-subunit. Only 2 family members 

Limited to Patient Descriptions, but Not in Untransfected TSA201 Cells (Negative Control). 

Figure 6. Na,ß3 expression in human ventricular myocardium. Rabbit anti-human SCN3B antibody detects bands in mem-

brae proteins from SCN3B transfected TSA201 cells (positive control) and from 2 human left ventricular myocardial prepara-

tions, but not in untransfected TSA201 cells (negative control).

Sodium Channel ß-Subunits and Arrhythmias 

Although mutations in Na1.1-ß-subunits have been associated with inherited diseases, including LQT3, BrS, progressive 

conduction disease, and atrial standstill,31 to date only 2 genes encoding sodium channel ß-subunits have been associated 

with human cardiac disease.7,32

It has long been appreciated that SCN5A mutations associated with LQTS and BrS are modulated by coexpression of 

SCN1B.33 BrS is known to be caused by a reduction in INa. 

The role of ß1-subunits to exacerbate the loss of function 

produced by R1232W/T1620 mol/L mutations in SCN5A in patients with BrS was demonstrated by Wan et al.34

Mutations in ß1-subunits (Na,ß1 and Na,ß1b) have recently been shown to be associated with a combined BrS and cardiac conduction disease phenotype in humans.7 Mutation in ß4-subunit have been reported to be associated with 

an increase in late INa giving rise to the LQT3 variant of the 

long-QT syndrome.32

In this study, we provide evidence that SCN3B is a 

BrS-susceptibility gene. An L10P missense mutation in a 

highly conserved cysteine residues, labeled C2, C21, C24, and C96 in ß1- and ß3-subunits.15 The disulfide bonds between C2 and 

C24, C21 and C96 are believed to correspond to the interaction 

site for ß3-subunit association. Moreover, the former bond is also responsible for forming the Ig fold for ß1 and ß3, a 

disruption of which can cause an inherited epilepsy syn-

drome. Our mutation (L10P) is located near C2, which may 

affect the interaction of the ß3-subunit with the sodium channel complex, and lead to the phenomenon that we 

observed. Little is known about cardiac sodium channel ß- and ß-subunits trafficking in vivo. A study using overexpression 
of fluorescent-tagged SCN5A, ß1, and ß2 in HEK293 cells suggested that SCN5A and ß2 are transported separately 

to the plasma membrane whereas SCN5A and ß1 form a complex in the endoplasmic reticulum that may facilitate 

plasma membrane trafficking.35 In PC12 and Chinese hamster ovary-K1/Na1.1.5 cells, enhanced GFP-tagged ßextracellular 
domain (extracellular domain deletion) ß3 mutant showed internal staining with little plasma membrane staining, and 

enhanced GFP-tagged ßinternal cardioverter defibrillator (intracellular domain deletion) ß3-mutant showed no evidence of 
surface staining but labeled an internal highly reticulated compartment that suggests endoplasmic reticulum. These 

results indicate that a mutation in the extracellular domain can impair trafficking of SCN3B to the membrane and that 
deletion of the intracellular domain totally disrupts trafficking of the ß-subunit. Our results suggest that WT ß3 plays a role 
in facilitating SCN5A transport to the plasma membrane, because a mutation in the extracellular domain of ß3 is 
capable of disrupting trafficking of SCN5A to the plasma membrane.

SCN3B and Cancer 

An interesting aspect of the family studied was that many 

family members died of lung cancer. SCN3B levels are upregulated in human cancer cell lines by DNA damage 

agents, as well as by overexpression of tumor suppressor p53, a transcription factor that induces growth arrest and/or apo-

ptosis in response to cellular stress. Introduction of the 

SCN3B gene into T98G and Saos2 cells potently suppressed colony formation, and adenovirus-mediated transfer of 

SCN3B induced apoptosis when combined with anticancer 

agents. These results suggest that SCN3B mediates a p53-
dependent apoptotic pathway and may be a candidate for 
gene therapy combined with anticancer drugs.36 A more 

recent study discovered that SCN3B is a candidate cancer gene, which could affect ion-channel transport.37 From 

the above point of view, it is tempting to speculate that the L10P mutation in SCN3B may also promote cancer and thus 

contribute to death of mutant carriers in this family. This 

hypothesis remains to be tested.

Limitations of the Study 

A genotype-phenotype correlation between the ß3-mutation and BrS phenotype is hampered by the high incidence of 
cancer deaths in this family, which may be caused by the same mutation in the ß3-subunit. Only 2 family members 

were available for study. A native American cohort was not available for the study as a control group.

Another limitation of the present study is the fact that 

characterization of the SCN3B mutation were carried out in a 

heterologous mammalian expression system, creating condi-

tions that may be different from those encountered in vivo as 

far as a contribution of ß2- or ß4-subunits, or other compo-

nents of the Na channel macromolecular complex. Despite 

these limitations, the electrophysiological characteristics of
the mutant channel are concordant with the BrS phenotype, and in combination with the clinical data supports a causal relationship between the L10P mutation in SCN3B and the disease.

Summary and Conclusion

Our results provide evidence in support of the hypothesis that mutations in the SCN3B-encoded Na,β3 subunit constitute another pathogenic mechanism responsible for development of the BrS phenotype secondary to a loss of function of cardiac sodium channel current.

Acknowledgments

We thank Judy Hefferon and Robert J. Goodrow, Jr, for technical assistance and Susan Bartkowiak for maintaining our genetic database.

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Disclosures

None.

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CLINICAL PERSPECTIVE
Brugada syndrome is an inherited cardiac arrhythmia syndrome associated with a high incidence of sudden cardiac arrest. This disorder has previously been linked to mutations in 6 different genes: SCN5A, GPD1L, CACNA1c, CACNB2b, SCN1B, and KCNE3. This study provides evidence that a mutation in SCN3B, encoding the β3-subunit of the sodium channel, can cause a loss of function in INa leading to a Brugada phenotype. The genes thus far associated with Brugada syndrome lead to either a loss of function in sodium or calcium channel current (INa and ICa) or to a gain of function in transient outward current potassium current (Ito). The decrease in inward current or increase in outward current cause a shift in the balance of current flowing during the early phases of the cardiac action potential leading to accentuation of the action potential notch in the epicardium but not in the endocardium. The resultant transmural gradient leads to an accentuation of the electrocardiographic J wave, manifest also as an ST-segment elevation, and the development of phase 2 reentry and polymorphic ventricular tachycardia. This Brugada syndrome phenotype is most commonly limited to the right precordial leads because Ito is usually most prominent in the right ventricular outflow tract. Understanding the genetic basis for the Brugada syndrome may assist with the diagnosis and ultimately with the approach to therapy.
A Mutation in the β3 Subunit of the Cardiac Sodium Channel Associated With Brugada ECG Phenotype


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