Brugada Syndrome Disease Phenotype Explained in Apparently Benign Sodium Channel Mutations

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Background—Brugada syndrome (BrS) is an arrhythmogenic disorder that has been linked to mutations in SCN5A, the gene encoding for the pore-forming α-subunit of the cardiac sodium channel. Typically, BrS mutations in SCN5A result in a reduction of sodium current with some mutations even exhibiting a dominant-negative effect on wild-type (WT) channels, thus leading to an even more prominent decrease in current amplitudes. However, there is also a category of apparently benign (atypical) BrS SCN5A mutations that in vitro demonstrates only minor biophysical defects. It is therefore not clear how these mutations produce a BrS phenotype. We hypothesized that similar to dominant-negative mutations, atypical mutations could lead to a reduction in sodium currents when coexpressed with WT to mimic the heterozygous patient genotype.

Methods and Results—WT and atypical BrS mutations were coexpressed in Human Embryonic Kidney-293 cells, showing a reduction in sodium current densities similar to typical BrS mutations. Importantly, this reduction in sodium current was also seen when the atypical mutations were expressed in rat or human cardiomyocytes. This decrease in current density was the result of reduced surface expression of both mutant and WT channels.

Conclusions—Taken together, we have shown how apparently benign SCN5A BrS mutations can lead to the ECG abnormalities seen in patients with BrS through an induced defect that is only present when the mutations are coexpressed with WT channels. Our work has implications for risk management and stratification for some SCN5A-implicated BrS patients. (Circ Cardiovasc Genet. 2014;7:123-131.)

Key Words: arrhythmias, cardiac ■ Brugada syndrome ■ electrophysiology ■ ion channels ■ SCN5A protein, human

Brugada syndrome (BrS) is a potentially fatal arrhythmogenic disorder characterized on the ECG by ST-segment elevation in the right precordial leads.1 Although afflicted patients have structurally normal hearts, they are predisposed to sudden cardiac death. It has been estimated that the syndrome is responsible for ≥20% of sudden cardiac death in patients with structurally normal hearts and ≥4% of all sudden deaths.2,3 BrS is typically inherited in an autosomal-dominant fashion, and currently mutations in ≥12 different genes have been implicated as causes of this disorder.4 Irrespective of the genes involved, the BrS ECG phenotype is catalyzed ultimately by an imbalance of the inward and outward currents during phase 1 of the cardiac action potential.2 Mutations in the SCN5A gene encoding the cardiac sodium channel Na1.5 are the predominant source of inherited BrS, accounting for ≥20% to 30% of all BrS cases.1

In general, in vitro experiments in heterologous expression systems show that SCN5A BrS mutations result in a major loss of sodium current and are thus able to explain the BrS phenotype of afflicted patients. Nevertheless, apparently benign SCN5A BrS mutations exist that do not exhibit this typical loss-of-function phenotype but rather display only small biophysical defects, if any. Consequently, defects in these atypical mutations seem insufficient to support the BrS ECG phenotype and explain the clinical manifestation of BrS in mutation carriers. This observation led us to question the nature of these mutations and ask how atypical SCN5A BrS mutations may cause a BrS phenotype despite near normal channel behavior.

Some typical (ie, loss of function) SCN5A BrS mutations have a dominant-negative effect on wild-type (WT) channels, therefore leading to an even more prominent decrease in sodium currents.5,6 Importantly, we have shown that the mechanism by which an SCN5A BrS mutation can produce a dominant-negative effect on the WT channel involves some level of interaction between 2 α-subunits.5 Moreover, work from our group and others has shown that a sodium channel polymorphism can modulate biophysical and trafficking defects in a variety of SCN5A mutations located on separate alleles.7,8 Finally, Tester et al9 reported a SCN5A mutation

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DOI: 10.1161/CIRCGENETICS.113.000292

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that, despite having normal physiological characteristics when expressed alone, produced a pathogenic effect when expressed in the presence of a common sodium channel polymorphism. Based on this information, we hypothesized that atypical BrS mutations may produce significant reductions in sodium currents when coexpressed with WT, thus explaining the manifestation of the disorder.

To mimic the heterozygous genotype present in patients, we coexpressed atypical SCN5A BrS mutations with WT channels and explored whether their biophysical and functional properties were modified. In fact, we found numerous atypical SCN5A BrS mutations that, although mainly innocuous and indistinguishable from WT channels when expressed alone, demonstrated significant reductions in total sodium current density when coexpressed with WT channels. The current reductions observed on coexpression explain the BrS disease phenotype, as it is similar in magnitude to what is observed for typical loss-of-function mutations. Importantly, we have unveiled how apparently benign SCN5A BrS mutations with minimal biophysical defects lead to an emergent loss of function as a result of interaction between mutant and WT channels. This mechanism reconciles the phenotype of atypical mutations with total sodium current amplitude and can explain the clinical manifestation of BrS seen in afflicted patients.

**Methods**

**Cloning of SCN5A Mutations**


**Expression of SCN5A in Heterologous Expression Systems**

Cardiac sodium channel was expressed using transient transfections of mutant SCN5A together with GFP either in human embryonic kidney cells (HEK293), Chinese hamster ovary (CHO) cells, neonatal rat ventricular myocytes (NRVMs), or iCell cardiomyocytes (Cellular Dynamics International, Madison, WI). Coexpression of WT was achieved using a cyan fluorescent protein (CFP)-tagged WT channel. Transfection of HEK293 and CHO cells was performed using the Polyfect Transfection Kit (Qiagen, Valencia, CA) according to the manufacturer’s protocol. The total DNA transfected was equal to 0.3 μg for both single and cotransfections (a 1:1 ratio was used for cotransfections). Transfection of either iCells or NRVMs was accomplished using Lipofectamine 2000 (Life Technologies, Grand Island, NY) according to the manufacturer’s protocol, using 1.2-μg DNA/35-mm dishes.

**Cellular Electrophysiological Measurements for Functional Characterization**

Sodium currents from transfected cells were recorded 1 day after transfection at room temperature (22°C–23°C) in the whole-cell configuration of the patch-clamp technique. Patch electrodes were prepared from 8161 Corning glass (Dow-Corning, Midland, MI), and the resistances of the electrodes were 1.5 to 2.5 MΩ. To minimize voltage-clamp errors, series resistance compensation of Axopatch 200A was performed to values >85%. To generate voltage-clamp command pulses, PCLAMP version 10 (Molecular Devices, Sunnyvale, CA) was used. The intracellular solution contained (in mmol/L, at pH 7.4) NaCl 35, CsF 105, EGTA 10, and Cs-HEPES 10. The external solution for HEK293 and CHO cells contained (mmol/L) NaCl 140, KCl 5, MgCl2 1, CaCl2 2, glucose 10, and HEPES 10 (pH 7.4). For NRVM and iCells, a low sodium external solution was used containing 35 mmol/L sodium.

Whole-cell I_{Na} densities and current–voltage relationships were recorded by holding the resting membrane potential at −120 mV and stepping in 10-mV intervals from −80 to +60 mV for 30 ms. Steady-state inactivation was elicited using 500 ms prepulses in the range of −140 to +60 mV in 10-mV increments followed by a 30-ms test pulse to −30 mV. Time course of recovery from inactivation (τ_{rec}) was studied using a 2-pulse protocol with a 30-ms prepulse to −30 mV with varying rest intervals at −120 mV, followed by a 30-ms test pulse to −30 mV.

Block by extracellular applications of [2-(trimethylammonium)ethyl] methanethiosulfonate bromide (MTSET; 2 μmol/L) was measured using a 30-ms test pulse to −30 mV. Percentage of block was obtained by comparing residual currents after 10 minutes of exposure to MTSET to currents before application of the drug.

**Data Analysis**

Data acquisition of voltage-clamp data was performed using Clampex 10 (Molecular Devices, Union City, CA). Off-line data analysis was performed with Clampfit 10 (Molecular Devices) and Origin 8.5.1 (OriginLab Corp, Northampton, MA). For recovery from inactivation, peak current amplitude was fit to the following equation:

\[
\frac{I_{\text{test}}}{I_{\text{pre-pulse}}} = 1 - \exp(-t/\tau_{\text{rec}})
\]

For steady-state inactivation, normalized currents were fit to a Boltzmann distribution:

\[
I / I_{\text{max}} = (1 + \exp(V - V_{1/2}) / k_T)^{-1}
\]

For measurements of current density, after entering whole-cell mode, a 10-nM pulse was administered to each cell to determine capacitation as area under the curve (pF). Experimentally determined capacitation was used to normalize currents evoked from each cell (pA/pF).

**Cell Surface Biotinylation**

A total of 3×100-mm dishes of HEK293 cells were transfected with 2.5-μg DNA for each separate construct per dish. Cells were collected 48 hours after transfection and washed 3× with PBS. Biotinylation was performed in 10-mL ice-cold PBS containing 0.25-mg/mL sulfo-NHS-SS-Biotin for 30 minutes at 4°C. A total of 10 mmol/mL glycine was added to quench the reaction. Cell lysis solution contained (in mmol/L) 50 HEPES (pH 7.4), 150 NaCl, 1.4 MgCl2, 1 EGTA, 10% glycerol, 1% triton X-100, and 1.2 mg/mL N-ethyl-maleimide with protease inhibitors. NeutrAvidin Agarose was used to pull down labeled proteins. Eluted proteins were then used for Western blotting as previously described11 and blotted using a sodium channel antibody (Millipore Polyclonal Anti-Na+ Channel III–IV loop). Pan-cadherin (Cell Signaling Technology) was used as a loading control for the cell surface biotinylated fraction and actin (Sigma-Aldrich monoclonal Anti-Actin, Clone AC-40) was used as a negative control for the cell surface biotinylated fraction. To determine the protein expression level, the sodium channel bands were normalized to the control bands (actin for total lysate and pan-cadherin for biotinylated fractions).

**Coimmunoprecipitation**

Coimmunoprecipitation experiments were performed using Dynabeads from Invitrogen (Life Technologies) as previously described.6 Briefly, washed magnetic beads were added to lysed HEK293 cells expressing the construct of interest. Immunoglobulin capture was performed for 60 minutes at room temperature (22°C–23°C) or overnight at 4°C using Protein G to control for nonspecific binding. Target-bound beads were then incubated for 30 minutes at 37°C and the supernant used for Western blot as previously described.11 An HA antibody (Roche monoclonal anti-HA high affinity
antibody) was used for the immunoprecipitation, and the blots were revealed with a GFP antibody (Clontech GFP monoclonal antibody).

**Neonatal Rat Myocytes**

Neonatal rat cardiac myocytes were isolated from 1- or 2-day-old Sprague-Dawley rats and cultured as previously described. Cells were transfected 48 hours after plating and used 24 hours after transfection for patch-clamp experiments. The neonatal rat procedures followed were in accordance with institutional guidelines.

**iCells Cardiomyocytes**

Induced cardiomyocytes from Cellular Dynamics were cultured at 37°C in 7% CO₂ according to the manufacturer's protocol. Transfections were performed using Lipofectamine 2000 as described above. Patch-clamp experiments were performed 24 to 36 hours after transfection.

**Statistical Analysis**

For sample sizes >10, statistical analysis was performed using the standard statistical package available in Origin 8.5.1 using parametric t tests with a critical value <0.05 considered significant after determining normality with the Shapiro–Wilk test. For sample sizes <10, the Mann–Whitney U test was performed using Minitab 16 Statistical Software.

**Results**

**Functional Analysis of Atypical BrS Mutations With and Without WT Channels**

Putative atypical mutations were selected from the Inherited Arrhythmias Database website (www.fsm.it/cardmoc). We first characterized the biophysical properties of these BrS mutations expressed alone and confirmed that they express currents largely indistinguishable from WT. We then expressed the so-called atypical BrS mutations with and without WT in HEK293 and recorded whole-cell currents. Figure 1A shows representative Na⁺ current traces (I₅) recorded from WT, the atypical mutation L567Q, and L567Q+WT. In these experiments, current density was similar for WT and L567Q (Figure 1B). However, on coexpression of L567Q with WT SCN5A, we observed a decrease in current density when compared with WT or L567Q alone (Figure 1A and 1B). Furthermore, our analysis showed no statistically significant difference in either steady-state inactivation or recovery from inactivation among WT, L567Q, and L567Q+WT (Table). Additional experiments were performed in CHO cells to exclude the possibility that our observations were restricted to channels expressed in HEK293 cells. Results were similar in CHO cells, with a reduction in current density on coexpression of L567Q with WT (Figure 1B).

**Functional Analysis of the Atypical Mutation, L567Q, in NRVM**

To determine whether the results observed in Figure 1 were also true for myocytes and not just an artifact of the expression system, NRVMs were transfected with either atypical L567Q or WT cDNA (Figure 2A). Although transfection of WT Na⁺,1.5 in NRVM produced the expected increase in sodium currents (Figure 2A and 2B), expression of L567Q in NRVMs produced a reduction in sodium currents, recapitulating the current reduction seen in HEK293 cells (Figure 2A and 2B). Thus, we conclude that the current reduction seen with the atypical mutant on coexpression with WT is not an artifact of HEK293 cells but is preserved in an endogenous cardiac background.

**Functional Analysis of Atypical Mutation, L567Q, in Human Cardiomyocytes**

Next we asked whether our results in NRVMs also hold true in human cardiomyocytes. In these experiments, we used iCell cardiomyocytes (Cellular Dynamics International, Madison, WI). These cells are human-induced pluripotent stem cell–derived cardiomyocytes of high purity and preserve many properties of native human cardiac myocytes. Figure 2C shows the current-voltage relationships obtained after transfecting either atypical mutant L567Q or GFP (control) in these human-induced pluripotent stem cell–derived cardiomyocytes. Current density was reduced when the atypical

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**Figure 1.** Effect of wild-type (WT) on L567Q atypical mutation. **A**, Representative traces for SCN5A-WT, SCN5A-L567Q, and SCN5A-L567Q+WT expressed in human embryonic kidney (HEK)-293 cells. **B**, Summary of peak current density in HEK293 and Chinese hamster ovary (CHO) cells showing a reduction in peak current density on coexpression with WT. Data presented as means±SEM (n=WT, 18; L567Q, 12; L567Q+WT, 14). Cells were transfected with 0.3-μg total DNA; 0.3 μg for mono-transfections or 0.15/0.15 μg for cotransfections (*P<0.05 compared with L567Q alone).
BrS mutation was expressed in these human cardiomyocytes compared with control transfections with GFP (Figure 2C). Again, our data are consistent with results obtained from NRVMs and HEK293 cells on heterologous expression of the respective mutant channels.

Identification of Additional Atypical BrS Mutations
To expand on our initial observations with L567Q, we screened for additional atypical BrS mutations. We found that N70K, E439K, G552R, E555K, A647D, R965H, E1053K, S1140T, L1501V, G1502S, and E1938K produced a reduction

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Table showing $V_{1/2}$ and $\tau_{rec}$ of mutations studied. $P$ values represent a comparison between mutation alone and mutation+WT. WT indicates wild-type.
in sodium current density on coexpression with WT (Figure 3A). On coexpression atypical mutations showed a reduction in peak current densities ranging from 30% to 70% when compared with mutations expressed alone (Figure 3A). As can be seen in the Table, the biophysical properties of all mutations studied, including recovery from inactivation (τ_{rec}) and steady-state inactivation (V_{1/2}) parameters, varied only minimally. Notably, in our screen we also identified atypical mutations with minimal defects whose current amplitude did not change on coexpression with WT in HEK293 cells (Figure 3B). To confirm these results obtained in HEK293 cells, we expressed in human cardiomyocytes 2 atypical mutations: E555K (which led to a reduction in total currents when coexpressed with WT) and T632M (which did not reduce currents when coexpressed with WT). We found, as expected, that total sodium current density was reduced in human cardiomyocytes when E555K was transfected in these cells (Figure 3B).

In marked contrast, when T632M was transfected in human cardiomyocytes, total sodium current densities were not significantly different from control transfections with GFP, hence confirming the results obtained in HEK293 cells (Figure 3C).

**Cell Surface Biotinylation and Coimmunoprecipitation**

The current reductions observed on coexpression of WT sodium channel with atypical BrS mutants may be attributed to either (1) a decrease in protein synthesis, or (2) an induced trafficking defect of WT and atypical mutant channels. To discriminate between WT and atypical mutant channels, we used a WT channel fused to yellow fluorescent protein, which increases the size of the channel protein. To assess changes in protein synthesis, WT-YFP and the atypical mutants L567Q and E555K were expressed either alone or on cotransfection. Figure 4A shows that total protein levels were maintained, which indicates that current reductions were not because of a decrease in protein synthesis. In a second step, cell surface biotinylation was performed to determine whether the level of channel proteins present at the cell surface membrane was modified. We found that WT, E555K, and L567Q showed similar protein levels at the cell surface, as expected based on similar current densities (Figure 4A). However, coexpression of WT with either atypical mutation drastically reduced cell surface expression of both WT and mutant channels (Figure 4A), suggesting that the reduction in currents is because of induced trafficking defects of both WT and mutant channels. As a negative control to demonstrate the purity for cell surface protein of our biotinylated fraction, the blots were also probed for the cytosolic protein actin. The absence of an actin signal in the surface fractions and the presence of an actin signal in the total cell lysate fractions confirmed the accuracy of the fractions. In addition, pan-cadherin was used as a loading control for the cell surface biotinylated fraction. We have shown previously that sodium channels associate with each other, which may explain the dominant-negative effect exerted by a BrS mutant on WT. Similarly, we used coimmunoprecipitation to test whether channel interactions were conserved between WT and atypical BrS mutants and found that this interaction was indeed maintained between WT and the atypical L567Q mutant (Figure 4B).

**Electrophysiological Hallmarks Coexpressing L567Q and WT**

Our surface biotinylation experiments demonstrated a drastic reduction in surface membrane protein of both WT and mutant...
channels, which in itself would explain the reduction in current amplitudes observed. However, surface biotinylation experiments do not guarantee that both channels are functional at the level of the cell surface membrane. Therefore, we tested the functionality of both mutant and WT sodium channels using a WT-C373Y construct resistant to block by MTSET. MTSET ordinarily inhibits sodium channels by binding to a cysteine at residue 373.13–15 Mutating residue C373 to tyrosine (C373Y) has been shown to essentially eliminate sensitivity to extracellular applications of MTSET.8 Thus, on coexpression with L567Q, selective mutation of WT-C373Y will render only WT current insensitive to MTSET.

Figure 5 shows representative sodium current recordings of WT-C373Y and L567Q in the absence and presence of 2 μmol/L of MTSET. After the addition of MTSET, drug effects were allowed to reach steady state before current measurements. As expected, WT-C373Y alone did not show any significant decrease in peak current density, whereas L567Q was blocked to a large extent (Figure 5A and 5B). More importantly, on coexpression MTSET reduced sodium current density by ≈50% at steady state (Figure 5A and 5B). As this decrease in peak current density can be attributed only to block of L567Q, our results suggest that both channels are functional at the cellular surface and present at similar levels.

Discussion

Mutations in the SCN5A gene encoding the cardiac sodium channel Na1.5 are implicated in multiple cardiac diseases, including fatal arrhythogenic disorders such as long QT syndrome type 3 and BrS. The BrS ECG phenotype is most often the result of loss of function in Na1.5. BrS typically manifests in vitro as a loss of whole-cell sodium currents and in vivo as conduction slowing. In marked contrast, we describe here a category of putative SCN5A BrS mutations, which are apparently benign (hence referred to as atypical mutations). These atypical mutations generally do not reduce current density in vitro as typical SCN5A BrS mutations do, and when they do, it is to such a small extent that they seem insufficient to produce a BrS phenotype. In addition, their biophysical properties of voltage–current relationship, steady-state inactivation, and recovery from inactivation remain relatively unchanged. However, we have now demonstrated that coexpression of many atypical mutations with WT SCN5A results in a counterintuitive decrease of current density. This decrease in current density is not the result of defective biosynthesis. Instead, our surface biotinylation experiments show a reduction in cell surface expression of both WT and mutant proteins on coexpression with WT channels. This is also reflected in our MTSET experiments showing that, although current density is reduced on coexpression, both WT and mutant channels are not only present but also functional. Taken together, our analysis suggests that the loss of current density on coexpression with WT is the result of an induced trafficking defect affecting both mutant and WT channels.

It is apparent that the mechanism governing the behavior of atypical BrS mutations relies on a damaging interaction...
between 2 otherwise normally functioning α-subunits. This observation is surprising because it deviates from the established paradigms of haploinsufficiency or negative dominance. To the best of our knowledge, no such mechanism has been reported previously. On a more mechanistic level, we speculate that there is (1) a misfolding event that occurs when a WT channel interacts with a mutant channel thereby impairing forward trafficking, (2) a symmetry-sensing event, which allows only for successful processing and export of like-channel pairs, or (3) increased degradation of both channels when coexpressed. However, these potential mechanisms are all hypothetical and need to be further explored.

Interestingly, the atypical mutations that displayed a loss of current density on coexpression with WT were located exclusively on the cytoplasmic domains of the channel (Figure 6, circles). This observation becomes more intriguing as one has to consider that Na$_{\alpha.1.5}$ is known to interact with a host of binding partners via its cytoplasmic domains, suggesting that the α-subunits may have interaction mediated by more than 1 of these binding partners. This represents other potential pathways through which 2 independently perfectly functional channels may become dysfunctional on coexpression. Theoretically, disabling the interaction between atypical BrS and WT sodium channels may restore trafficking and ameliorate the disease phenotype clinically, as channel function is normal in isolation. Further work will seek to examine the role of these interacting proteins on producing the BrS loss-of-function phenotype from atypical BrS mutations.

Our findings seem to contrast a recent report by Mercier et al., who found that for a dominant-negative mutation located on the extracellular loop of DIII, the β1-subunit seemed to be required for α–α interaction. However, our biochemical and patch-clamp experiments here show interaction between α-subunits without cotransfection of β1-subunits, and we have also demonstrated previously that coimmunoprecipitation between sodium channels was possible without β1-subunit overexpression. Despite extensive debate within the sodium channel community, the physiological effects of the β1-subunit remain poorly understood. Further complicating matters is the fact that HEK293 cells are known to endogenously express β1-subunits in a highly variable manner. Therefore, one potential explanation for the discrepancy between our work and that of Mercier et al could be because of different endogenous levels of β1-subunits present in the expression systems used.

A loss of function in SCN5A arising directly from the interaction of a disease allele with WT is attractive as it offers an elegant solution for apparently innocuous BrS mutations showing WT-like behavior. However, not all mutations without loss of function characteristics showed a current reduction

![Figure 4](image_url)  
**Figure 4.** A. Surface biotinylation experiments. Whole-cell lysate (left) and surface biotinylation fraction (right) for SCN5A-wild-type (WT)-YFP (yellow fluorescent protein) (2.5 μg), SCN5A-L567Q (2.5 g), SCN5A-WT-YFP (2.5 g)+SCN5A-L567Q (2.5 g), SCN5A-E555K (2.5 g), and SCN5A-WT-YFP (2.5 g)+SCN5A-E555K (2.5 g). The Western blotting was performed with a sodium channel antibody. The SCN5A-WT-YFP construct's size was ≈260 kDa, whereas the mutations constructs were ≈220 kDa. The cell surface biotinylation showed a dramatic reduction of both WT and atypical mutations on coexpression, whereas the total protein level was not modified. Actin was used as a negative control for the cell surface fractions, and pan-cadherin was used as loading control for the cell surface fractions. B. Coimmunoprecipitation experiments were performed between WT-YFP and a WT channel expressing an HA tag (WT-HA) and L567Q-YFP and WT-HA. The pulldown was performed with an HA antibody, and the blot was revealed with a GFP antibody. For both A and B, the figures are a representative example of ≥3 separate experiments.

![Figure 5](image_url)  
**Figure 5.** Both wild-type and mutant channels are functional at the plasma membrane. A. Sodium currents before and after the addition of 2 μmol/L [2-((trimethylammonium)ethyl] methanethiosulfonate bromide (MTSET) for SCN5A-C373Y (no reduction), SCN5A-L567Q (near total reduction), and SCN5A-L567Q+SCN5A-C373Y (≈50% reduction). B. Averaged percent current reduction. There was no reduction in SCN5A-C373Y (n=10), a near complete reduction in SCN5A-L567Q (n=15), and a 58% reduction in SCN5A-L567Q+SCN5A-C373Y (n=15). These results demonstrate that both channels are present at the cell surface in a similar ratio (*P<0.05 compared with SCN5A-C373Y).
on coexpression with WT. One possible explanation is the uncertainty of these mutations being benign polymorphisms instead of true BrS mutations. Importantly, although these SCN5A variants have been putatively found in patients with BrS, several of them have only been reported as candidate gene mutations in the Inherited Arrhythmias Database (www.fsm.it/cardmoc). Thus, there is a distinct possibility that these mutations are not truly the source of the disease, especially when we consider the fact that no obvious functional defects have been reported on coexpression with WT in a heterologous system or in human cardiomyocytes.

Most importantly, however, our study demonstrates that SCN5A mutations may not only affect their own expression but also reduce expression of WT/mutant channel complexes in a heterozygous situation. Overall, our results have a high level of clinical significance in that they offer a novel mechanism to understand the complex phenomenon of genotype–phenotype discordance that was presented by these atypical mutations, because up until now it was unclear how an SCN5A mutant channel with no major biophysical defects could lead to BrS. This genotype–phenotype discordance is of high interest to clinicians as it affects risk stratification and ultimately treatment options for patients with BrS and closely related family members who are mutation carriers. Although some progress has been made toward explaining the genotype–phenotype discordance of BrS (namely, the contribution of disease-modifying genes), it remains poorly understood. To this end, our present study identifies for the first time a pathophysiological mechanism for several atypical BrS SCN5A mutations and offers guidance for future treatment approaches similar to typical loss-of-function SCN5A BrS mutations.

**Sources of Funding**

This work was supported by an American Heart Association Scientist Development Grant (0635295 N; Dr Deschênes), National Institutes of Health R01 (HL094450; Dr Deschênes), an American Heart Association Pre-Doctoral Fellowship from the Great Rivers Affiliate 0815479D (Dr Shinlapawittayatorn), an American Heart Association Pre-Doctoral Fellowship from the Great Rivers 12PRE11940047 (M. Hoshi), and the Thailand Research Fund TRF-CHE Research Grant for New Scholar MRG5580125 (Dr Shinlapawittayatorn).

**References**


**CLINICAL PERSPECTIVE**

Brugada syndrome (BrS) is a potentially lethal genetic disease of cardiac rhythm that displays a high degree of variable penetrance. The only proven treatment is implantation of a cardioverter-defibrillator, which is costly and carries its own risks. Thus, appropriate risk stratification of affected lineages is critical. Mutations in the SCN5A gene encoding the cardiac sodium channel have been implicated in BrS. To lead to a BrS phenotype, mutations in SCN5A most often result in a loss of function of the sodium channel, leading to a drastic reduction in sodium currents. In marked contrast, however, we describe here a category of putative SCN5A BrS mutations, which are apparently benign (hence atypical mutations). These atypical mutations do not reduce current density in vitro, and it was therefore unclear how they could lead to a BrS phenotype. Here, we show how several previously benign-appearing SCN5A mutations can produce a BrS phenotype through a reduction in sodium currents when expressed at the heterozygous state. This work should aid the clinician in decision making when planning treatment for SCN5A-linked patients with BrS because we have now identified a pathophysiological mechanism for several previously apparently benign BrS SCN5A mutations. These results will offer guidance for future treatment approaches similar to typical loss-of-function SCN5A BrS mutations.
Brugada Syndrome Disease Phenotype Explained in Apparently Benign Sodium Channel Mutations
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Circ Cardiovasc Genet. 2014;7:123-131; originally published online February 26, 2014;
doi: 10.1161/CIRCGENETICS.113.000292

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