SCN5A Polymorphism Restores Trafficking of a Brugada Syndrome Mutation on a Separate Gene

Steven Poelzing, PhD; Cinzia Forleo, MD, PhD; Melissa Samodell, BS; Lynn Dudash, BS; Sandro Sorrentino, PhD; Matteo Anaclerio, MD, PhD; Rossella Troccoli, MD; Massimo Iacoviello, MD; Roberta Romito, MD; Pietro Guida, MS; Mohamed Chahine, PhD; Mariavittoria Pitzalis, MD, PhD; Isabelle Deschênes, PhD

Background—Brugada syndrome is associated with a high risk of sudden cardiac death and is caused by mutations in the cardiac voltage-gated sodium channel gene. Previously, the R282H-SCN5A mutation in the sodium channel gene was identified in patients with Brugada syndrome. In a family carrying the R282H-SCN5A mutation, an asymptomatic individual had a common H558R-SCN5A polymorphism and the mutation on separate chromosomes. Therefore, we hypothesized that the polymorphism could rescue the mutation.

Methods and Results—In heterologous cells, expression of the mutation alone did not produce sodium current. However, coexpressing the mutation with the polymorphism produced significantly greater current than coexpressing the mutant with the wild-type gene, demonstrating that the polymorphism rescues the mutation. Using immunocytochemistry, we demonstrated that the R282H-SCN5A construct can traffic to the cell membrane only in the presence of the H558R-SCN5A polymorphism. Using fluorescence resonance energy transfer and protein fragments centered on H558R-SCN5A, we demonstrated that cardiac sodium channels preferentially interact when the polymorphism is expressed on one protein but not the other.

Conclusions—This study suggests a mechanism whereby the Brugada syndrome has incomplete penetrance. More importantly, this study suggests that genetic polymorphisms may be a potential target for future therapies aimed at rescuing specific dysfunctional protein channels. (Circulation. 2006;114:368-376.)

Key Words: amino acids — electrophysiology — genes — ion channels — sodium

Brugada syndrome (BrS) is an inherited disorder characterized by ST-segment elevation in right precordial leads and increased susceptibility to ventricular arrhythmias and sudden cardiac death. The disease has been linked to the presence of mutations in the α-subunit of the human cardiac sodium channel (hNav1.5). Many previously identified mutations result in a loss of whole-cell sodium current. Earlier studies demonstrated that a single mutation in SCN5A, the gene encoding hNav1.5, is sufficient to cause the disease. In some cases, however, multiple mutations on SCN5A, which are by themselves benign, can cause the disease. Although BrS is an autosomal dominant disease, it is characterized by incomplete penetrance, a phenomenon still incompletely understood. Therefore, although the mutation is phenotypically expressed in most individuals with the mutation, there are individuals in families with BrS who have a mutation but are asymptomatic.

Editorial p 360
Clinical Perspective p 376

One specific SCN5A missense mutation (R282H-SCN5A) was first identified by Priori et al in a group of patients with BrS diagnosed by ST-segment elevation in right precordial leads at baseline or during administration of a sodium channel blocker. This mutation, resulting in histidine replacing arginine at amino acid 282 of hNav1.5, was found on 1 allele of a 37-year-old patient (patient II-2) with a positive BrS flecainide-induced ECG pattern. This individual became the proband for a subsequently tested and genotyped family (Figure 1A). The R282H-SCN5A mutation occurs in domain 1 of hNav1.5 in the beginning of the pore-forming loop (SS1) (Figure 1B). Importantly, the patient’s daughter, who was asymptomatic and did not have a typical BrS ECG pattern either at baseline or after administration of flecainide (patient III-1), was a carrier of the R282H-SCN5A mutation. Further genotyping determined that the subject’s second allele

© 2006 American Heart Association, Inc.
The R282H-SCN5A, H558R-SCN5A, and C373Y mutations were created in the hNa1.5 protein fragments expressed in the pEFP-N3 or pEYFP-N3 vector (BD Biosciences Clontech).

**Expression of hNav1.5 in HEK293 Cells**

Transient transfections of SCN5A expressed in GFP-RES were performed in human embryonic kidney cells (HEK293) with the Polyfect transfection kit (Qiagen, Valencia, Calif) according to the manufacturer’s protocol for 24 hours.

**Electrophysiology**

Macroscopic sodium currents from transfected cells were recorded using the whole-cell configuration of the patch-clamp technique as previously described. Cells that emitted green fluorescence and expressed I\textsubscript{Na-like} currents were considered to express mutants and/or polymorphisms of hNav1.5. Patch electrodes were made from 8161 Corning glass (Dow-Corning, Midland, Mich). Low-resistance electrodes (<2 MΩ) were used, and a routine-series resistance compensation of an Axopatch 200A was performed to values >80% to minimize the voltage-clamp errors. Voltage-clamp command pulses were generated by a microcomputer using PCLAMP software version 9.02 (Axon Instruments, Foster City, Calif). To stabilize the current, experiments were performed 10 minutes after entering whole-cell configuration. Membrane currents were filtered at 5 kHz and digitized with 12-bit resolution. The internal solution contained (in mmol/L) NaCl 35, CsF 105, EGTA 10, and Cs-HEPES 10 adjusted to pH 7.4. The bath solution contained (in mmol/L) NaCl 140, KCL 5, MgCl\textsubscript{2} 1, CaCl\textsubscript{2} 2, HEPES 10, and glucose 10 adjusted to pH 7.4. Experiments were performed at room temperature (22°C to 23°C).

Whole-cell sodium current densities were made by holding the resting membrane potential at −120 mV and stepping in 10-mV intervals from −80 to 30 mV. Time course of recovery from inactivation (\(\tau_{\text{recovery}}\)) was studied using a 2-pulse protocol with a 50-ms prepulse to −30 mV with varying rest intervals at −120 mV, followed by a 30-ms test pulse to −30 mV. Peak current amplitude was fit to the following equation:

\[
I_{\text{peak}}/I_{\text{pre-pulse}} = 1 - \exp(-t/\tau_{\text{recovery}})
\]

Voltage dependence of steady-state inactivation was determined by 500-ms prepulses ranging from −140 to −30 mV. Peak current was fit to a Boltzmann distribution:

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

**Image Analysis and Calculation of Fluorescence Resonance Energy Transfer Ratios**

Images were acquired with an Olympus IX71 fluorescence microscope that was fitted with a Hamamatsu ORCA-ER charge-coupled device (12 bit) and controlled by the SLIDEBOOK software package from Intelligent Imaging Innovations (Denver, Colo). Filter-cube specifications for the fluorescent channels were as follows for excitation and emission, respectively: enhanced cyan fluorescent protein (ECFP), 430±25 and 470±30 nm; enhanced yellow fluorescent protein (EYFP), 500±20 and 535±30 nm; and fluorescence resonance energy transfer (FRET), 430±25 and 535±30 nm. The beam splitter was the Chroma 86002v2bs multiband beam splitter for ECFP and EYFP (Rockingham, Vt).

Image analysis involved 3 basic operations: subtraction of background autofluorescence and blurred light, quantification of fluorescence intensity, and calculation of a corrected FRET (FRETc) calculated by the following equation:

\[
FRETc = I_{\text{donor}} - dI_{\text{AX}} - dI_{\text{AA}}/I_{\text{AX}}
\]

where \(I_{\text{AX}}\) is the fluorescence intensity from the FRET filter set and \(I_{\text{donor}}\) and \(I_{\text{AA}}\) are the fluorescent intensities from ECFP (the donor) and EYFP (the acceptor), respectively. The cross-talk coefficients a

---

**Methods**

**Genotyping**

Molecular analyses on the SCN5A gene were performed according to our previous article. Our local Ethics Committee approved the study, and written informed consent was obtained from the participants.

**Cloning of SCN5A Mutants and Polymorphisms**

The R282H-SCN5A, H558R-SCN5A, and C373Y mutations were created in the hNa1.5 background (PubMed Accession No. NM 198056) expressed in the GFP-RES vector (BD Biosciences Clontech, San Jose, Calif) using the Stratagene QuikChange XL Site-Directed Mutagenesis Kit. R282H-SCN5A-FLAG was generated as previously described. hNa1.5 protein fragments were expressed in the pEFP-N3 or pEYFP-N3 vector (BD Biosciences Clontech).

---

**Figure 1.** A, Pedigree of a family with BrS. The solid symbols represent the individuals clinically identified with the disease. Individual II–1 is asymptomatic and does not show the typical BrS ECG pattern despite having the R282H-SCN5A mutation. That individual also has an H558R-SCN5A polymorphism, which is located in the intracellular domain 1 to 2 linker (Figure 1B). Therefore, we hypothesized that the H558R-SCN5A polymorphism could suppress or attenuate expression of BrS ECG alterations related to the R282H-SCN5A mutation by rescuing the mutation. The purpose of this report is 3-fold: (1) to demonstrate that coexpressing the R282H-SCN5A mutation and the H558R-SCN5A polymorphism produces fully functional sodium currents similar to control, (2) to assess alterations related to the R282H-SCN5A mutation by rescuing the H558R-SCN5A polymorphism and (3) to investigate the mechanism by which the H558R-SCN5A polymorphism rescues the R282H-SCN5A mutation.
and d are considered constant. The corrected FRET ratio was defined as $FRET_c / I_{DD}$.

**Immunocytochemistry**

Transfected cells were permeabilized with 0.1% Triton into 1 mmol/L PBS/0.5% BSA solution before antibody incubation. Cells were fixed with an acetone/methanol solution (1:3) for 20 minutes. The primary antibody was a mouse anti-FLAG M2 (1:4000) (Stratagene, La Jolla, Calif). The secondary antibody was a conjugated AffiniPure goat anti-mouse (1:250) (Molecular Probes, Eugene, Ore). Confocal fluorescent images were obtained with a Leica laser scanning confocal microscope ($\times40$ oil immersion lens, airy 1 pinhole).

**Statistical Analysis**

Statistical analysis of the data were performed with a 2-tailed Student $t$ test for paired and unpaired data or a single-factor ANOVA. A value of $P<0.05$ was considered statistically significant. All values are reported as mean±SD unless otherwise noted.

The authors had full access to the data and take responsibility for their integrity. All authors have read and agree to the manuscript as written.

**Results**

**Effect of Coexpressed H558R-SCN5A and R282H-SCN5A**

To determine whether coexpressing the H558R-SCN5A polymorphism with the R282H-SCN5A mutation produces fully functional currents similar to control, sodium currents were recorded from HEK293 cells transfected with wild-type (WT)-SCN5A, H558R-SCN5A, R282H-SCN5A, or a combination of H558R-SCN5A and R282H-SCN5A sodium channels produced on an hNav1.5 background (PubMed Accession No. NM 198056). Cells were transfected and incubated for 24 hours at 37°C. Figure 2A shows representative current traces from cells transfected with WT-SCN5A ($2\mu g$). When the R282H-SCN5A mutation was expressed alone ($2\mu g$ R282H-SCN5A DNA), little to no whole-cell sodium current was recorded (Figure 2A). The lack of whole-cell currents produced by the R282H-SCN5A mutation is consistent with other BrS mutations.3–7 The R282H-SCN5A mutation (1 $\mu g$) coexpressed with WT-SCN5A (1 $\mu g$; $n=6$) significantly reduced peak current by ~45% compared with WT-SCN5A (2 $\mu g$). H558R-SCN5A (0.5 $\mu g$) and R282H-SCN5A (1.5 $\mu g$; $n=23$) reduced peak current by 54% compared with WT-SCN5A (2 $\mu g$; *$P<0.05$ vs WT-SCN5A; †$P<0.05$). Error bars expressed as SD.
SCN5A produced significantly less peak current densities affecting cells with either 1

The whole-cell peak current density of heterologously expressed proteins often is criticized because it assumes a correlation between the amount of DNA transfected and the amount of current and/or protein produced. Therefore, the apparent restoration of R282H-SCN5A by H558R-SCN5A could be a result of greater transfection efficiency of H558R-SCN5A and reduced efficiency of R282H-SCN5A. Transfected cells with either 1 μg WT-SCN5A or 1 μg H558R-SCN5A produced significantly less peak current densities (−322±28 and −332±34 pA/pF, respectively) compared with 2 μg WT-SCN5A (−595±55 pA/pF), which corresponds to a 45% reduction in peak current density (Figure 2C). These results suggest that whole-cell peak current densities of H558R-SCN5A and WT-SCN5A are related to the amount of DNA transfected.

The β1-subunit also may influence protein trafficking as suggested by Biskup et al. However, coexpressing the β1-subunit with WT-SCN5A or H558R-SCN5A did not significantly alter the principal finding of the study that only H558R-SCN5A was capable of restoring the function of the R282H-SCN5A mutation (data not shown).

The whole-cell peak current density of heterologously expressed proteins often is criticized because it assumes a correlation between the amount of DNA transfected and the amount of current and/or protein produced. Therefore, the apparent restoration of R282H-SCN5A by H558R-SCN5A could be a result of greater transfection efficiency of H558R-SCN5A and reduced efficiency of R282H-SCN5A. Transfected cells with either 1 μg WT-SCN5A or 1 μg H558R-SCN5A produced significantly less peak current densities (−322±28 and −332±34 pA/pF, respectively) compared with 2 μg WT-SCN5A (−595±55 pA/pF), which corresponds to a 45% reduction in peak current density (Figure 2C). These results suggest that whole-cell peak current densities of H558R-SCN5A and WT-SCN5A are related to the amount of DNA transfected.

### Concentration of H558R-SCN5A Determines R282H-SCN5A Rescue

To further verify the correlation between the amount of DNA transfected and current density and to determine the relationship of R282H-SCN5A rescue by H558R-SCN5A, 1.5 μg R282H-SCN5A was coexpressed with 0.5 μg H558R-SCN5A. Peak current density was reduced by 54.8% to −231±22 pA/pF ($P = 9\times10^{-5}$) under these conditions (Figure 2C). These data suggest that there is a 1:1 ratio between the concentration of H558R-SCN5A channels and R282H-SCN5A channels because when the amount of H558R-SCN5A is reduced by half, the peak current density decreases by ~50% regardless of increases in R282H-SCN5A. These data also are consistent with the aforementioned findings in which 1 μg H558R-SCN5A and 1 μg R282H-SCN5A produced peak current densities similar to 2 μg WT-SCN5A. Therefore, these data suggest that the H558R-SCN5A protein interacts with the R282H-SCN5A protein because the concentration of H558R-SCN5A protein is the limiting factor underlying the peak whole-cell current density.

### R282H-SCN5A Protein Trafficking

To determine whether the R282H-SCN5A mutation is a functional or trafficking mutation, the R282H mutation was created on the SCN5A-FLAG construct in which the tag epitope FLAG was inserted in the S5-to-S6 extracellular loop of domain 1. Confocal immunofluorescence revealed that R282H-SCN5A-FLAG staining produces protein localized in internalized compartments of a representative cell (Figure 3A). Likewise, coexpressing WT-SCN5A + R282H-SCN5A-FLAG revealed FLAG staining in internalized compartments, consistent with the inability of WT-SCN5A to rescue R282H-SCN5A. Importantly, coexpressing H558R-SCN5A + R282H-SCN5A-FLAG resulted in significant FLAG staining in internalized compartments of the cell and around the perimeter of the cell (Figure 3C). Summary data in Figure 3D demonstrate that the average fluorescence intensity per unit area (Figure 3C). Summary data in Figure 3D demonstrate that the average fluorescence intensity per unit
area in the membrane divided by the average fluorescence intensity per unit area in intracellular compartments (membrane to intracellular fluorescence ratio) is significantly reduced in the R282H-SCN5A and WT-SCN5A+R282H-SCN5A-FLAG groups compared with the H558R-SCN5A+R282H-SCN5A-FLAG group (n=10 for all groups; \( P=6\times10^{-6} \)), consistent with R282H-SCN5A rescue by H558R-SCN5A. These data demonstrate that R282H-SCN5A-FLAG produces a trafficking-deficient protein that is rescued only when H558R-SCN5A also is present in the cell. However, it was still unclear whether the rescued R282H-SCN5A produced functional protein in the membrane.

A complementary approach was developed to determine whether channels with the R282H-SCN5A mutation were trafficking to the membrane and functional in the presence of the H558R-SCN5A polymorphism. A second mutation (C373Y) was added to WT-SCN5A channels. Changing the hNav1.5 amino acid 373 from cysteine to tyrosine is expected to decrease channel sensitivity to extracellular application of [2-(trimethylammonium) ethyl] methanethiosulfonate bromide (MTSET), which blocks channels by binding to cysteine at residue 373. Using MTSET reagents to bind to pore cysteines is an established technique for preferentially blocking sodium channels and other channels such as Kir2.1 potassium channels to assess for their presence and functionality of the channel in the cell membrane. C373 is located in domain 1 of hNav1.5 in the pore-forming loop (SS2) before S6 (Figure 1B). Representative recordings of whole-

**Figure 3.** R282H-SCN5A is a trafficking mutation. A, Top, Confocal immunofluorescence reveals R282H-SCN5A-FLAG staining in internalized compartments of a cell transfected with R282H-SCN5A-FLAG (red signal). Bottom, Z-scan image from the dashed white line in the top panel demonstrates FLAG staining within intracellular compartments. B, Cells transfected with WT-SCN5A+R282H-SCN5A-FLAG reveal FLAG staining also in internalized compartments. C, H558R-SCN5A+R282H-SCN5A-FLAG reveals strong FLAG staining in the cell membrane in an X-Y confocal image (top) and Z-scan image (bottom) from the white dashed line in the top panel. D, Summary data demonstrate that R282H-SCN5A-FLAG membrane staining is significantly reduced compared with H558R-SCN5A+R282H-SCN5A-FLAG (n=10 for each group; \(* P<0.0001\)).

**Figure 4.** The H558R-SCN5A polymorphism rescues the R282H-SCN5A mutation by increasing R282H-SCN5A membrane trafficking. A, MTSET application blocks all WT-SCN5A and reduces peak current of WT-SCN5A+C373Y-SCN5A and H558R-SCN5A+R282H-C373Y-SCN5A channels. B, MTSET blocks almost all WT-SCN5A (n=6), H558R-SCN5A (n=5), and WT-SCN5A+R282H-C373Y-SCN5A (n=4) channels. MTSET does not block C373Y-SCN5A channels (n=4). Whole-cell current is reduced by 50% during application of MTSET to WT-SCN5A+C373Y-SCN5A (n=5) and H558R-SCN5A+R282H-C373Y-SCN5A (n=12). Error bars expressed as SD.
cell sodium currents in Figure 4A demonstrate the effects of 1 μmol/L MTSET on WT-SCN5A, WT-SCN5A+C373Y-SCN5A, and H558R-SCN5A + R282H-C373Y-SCN5A channels. MTSET blocked all the current in cells expressing WT-SCN5A hNav1.5 protein alone because the MTSET could bind to the cysteine at position 373. This result is summarized in Figure 4B, which demonstrates that MTSET blocks 98±4% of WT-SCN5A channels (2 μg). These data are consistent with previous findings that MTSET preferentially blocks WT-SCN5A channels with C373.15–17 Measurements were performed at 5 and 10 minutes after MTSET application to determine whether peak current density was measured at steady state. There were no significant differences in peak current density at 5 and 10 minutes after MTSET application, demonstrating that MTSET block reached its maximal effect after 5 minutes (data not shown).

As expected, MTSET does not significantly decrease peak current densities in sodium channels with only the C373Y-SCN5A mutation (2 μg) (C373Y-SCN5A; Figure 4B). Importantly, when WT-SCN5A channels (1 μg) are coexpressed in a 1:1 ratio with channels containing the C373Y-SCN5A mutation (1 μg), MTSET significantly reduced whole-cell sodium current by 42.6±9.5% (paired comparison, P=0.006) after 10 minutes (Figure 4A and 4B). This decrease in peak current density is due to the blockade of WT-SCN5A channels. Furthermore, MTSET blocked nearly all whole-cell sodium currents in cells expressing H558R-SCN5A alone (2 μg) by 90.6±11.7% (P=3×10⁻⁷) after 10 minutes (Figure 4B). These data are further consistent with MTSET preferentially blocking functional sodium channels with a cysteine at amino acid residue 373.

We created a construct to produce hNav1.5 channels with both R282H and C373Y mutations (cis) on the same protein (R282H-C373Y-SCN5A). We hypothesized that if the H558R-SCN5A polymorphism rescues the R282H-SCN5A mutation by allowing the R282H-SCN5A mutant protein to traffic to the membrane, where it is functional, then MTSET should decrease peak sodium current density by 50% in cells coexpressing H558R-SCN5A and R282H-C373Y-SCN5A channels. MTSET significantly reduces whole-cell sodium current by 48.1±10.0% after 10 minutes (P=0.003; Figure 4A and 4B) in cells coexpressing H558R-SCN5A (1 μg) and R282H-C373Y-SCN5A channels (1 μg) (H558R-SCN5A+R282H-C373Y-SCN5A). Furthermore, MTSET blocked nearly all current (reduction of 86±8% in cells coexpressing 1 μg WT-SCN5A and 1 μg R282H-C373Y-SCN5A channels (WT-SCN5A/R282H-C373Y-SCN5A; Figure 4B). These data demonstrate that hNav1.5 channels with the R282H-SCN5A mutation are functionally expressed in the plasma membrane in the presence of hNav1.5 channels with the H558R-SCN5A polymorphism but not in the presence of WT-SCN5A channels. MTSET eliminated only 50% of the currents when R282H-C373Y-SCN5A was coexpressed with H558R-SCN5A compared with almost all the currents when R282H-C373Y-SCN5A was coexpressed with WT-SCN5A. Therefore, MTSET predominantly blocked the H558R-SCN5A channels when coexpressed with R282H-C373Y-SCN5A channels because H558R-SCN5A channels maintain C373 in the protein sequence. The current remaining after application of 1 μmol/L MTSET was measured to determine the biophysical properties of the R282H-SCN5A mutated channel. The recovery and steady-state inactivation characteristics of the remaining R282H-C373Y-SCN5A channels were similar to control, suggesting that the R282H-SCN5A mutation is trafficking deficient but not functionally deficient in the membrane (the Table).

Mechanism of R282H-SCN5A Rescue by H558R-SCN5A
To determine whether the mechanism by which H558R-SCN5A rescues R282H-SCN5A is direct interaction of α-subunits, small hNav1.5 protein fragments centered on H558 fused to CFP or YFP were used for FRET studies. Three-channel FRET ratios were normalized to CFP and reported as FRETc as previously described by Vanderklift and colleagues.19 Representative FRET recordings are shown in Figure 5. Fragments centered on 558 (40 amino acids) were fused to either CFP or YFP (Figure 5). When cells coexpressed homotypic amino acid fragments (R558-CPF+, R558-YFP+), or H558-CPF+H558-YFP+), there was little to no FRETc, as demonstrated in Figure 5A and 5B. Cells coexpressing R558-CPF+, H558-YFP had a 3-fold increase in FRETc values compared with the homotypic FRETc values specifically within internalized compartments of the cell (0.04±0.02 and 0.01±0.01, respectively; P=0.006; Figure 5C). In all experiments, FRET was absent from the cell membrane, which is consistent with the inability of these protein fragments to traffic to the membrane. These data suggest that the interaction of the hNav1.5 protein first occurs within intracellular compartments of the cell before protein trafficking to the membrane. More importantly, hNav1.5 interaction occurs preferentially when 1 protein expresses R558 and the other expresses H558.

Discussion
Mutations of the SCN5A gene underlie multiple cardiac diseases such as the long-QT syndrome type 3 and BrS. Although the long-QT syndrome type 3 is most often associated with a gain in sodium channel function, BrS is associated with a loss of whole-cell sodium channel current and thereby manifests as a slow-conduction phenotype. Importantly, it is well documented that BrS is an autosomal dominant disease with variable penetrance.20–23 However, little is known of the mechanisms that underlie this variable penetrance. Baroudi et al8 demonstrated that the individual BrS mutations R1232W-SCN5A and T1620M-SCN5A each produced functional sodium channels with biophysical properties significantly different from WT-SCN5A. However, the combination of the 2 mutations on the same gene (R1232W-T1620M-SCN5A) blocked protein trafficking of the channel. Importantly, the Baroudi et al study offers a mechanism for explaining the severity of the disease but does not explain the mechanism of penetrance.

To the best of our knowledge, this is the first study to suggest that the penetrance of BrS can be explained by a polymorphism on an allele separate from that with the mutation. Furthermore, the mutation is fully rescued by a polymorphism. Importantly, instead of producing a loss of
protein trafficking, this study suggests that the protein with the polymorphism restores the function of the mutant protein by interacting with the mutant before it traffics to the membrane.

The penetrance of BrS is better studied with larger multiplex families and calls into question whether the proposed mechanism is the only explanation for incomplete penetrance of the patient with the R282H-SCN5A mutation and the H558R-SCN5A polymorphism. Although this study does not prove the mechanism of the incomplete penetrance in patient III-1 (Figure 1A), the in vitro studies strongly suggest that the H558R-SCN5A polymorphism restores the function of the R282H-SCN5A mutation and is consistent with the current phenotype of the asymptomatic patient (patient III-1) and her negative response to the flecainide challenge. Specifically, cells coexpressing equal amounts of R282H-SCN5A and H558R-SCN5A have peak current densities similar to an equal total amount of WT-SCN5A. When the concentration of H558R-SCN5A was reduced and coexpressed with an increased concentration of R282H-SCN5A, the peak current density was reduced, implying that the concentration of H558R-SCN5A is the limiting rescuing reagent. H558R-SCN5A also restores membrane trafficking of R282H-SCN5A-FLAG, as evidenced by confocal immunocytochemistry. Furthermore, we demonstrate that the R282H-C373Y-SCN5A mutation is rescued by H558R-SCN5A and that MTSET application reduces peak current density by 50% in cells transfected with equal amounts of R282H-C373Y-SCN5A+H558R-SCN5A. On the other hand, MTSET blocks almost all current in cells transfected with SCN5A that retains C373 and does not significantly change peak current with C373Y-SCN5A. This is strong evidence that the mutated channel is able to traffic to the membrane and produces a functional channel in the presence of the polymorphism. Although the in vitro results present a line of evidence consistent with the phenotype observed in the family depicted in Figure 1A, we cannot be sure that this is the exact mechanism of incomplete penetrance because these experiments are conducted in a heterologous expression system.

It is important to note that the H558R polymorphism alone produced fully functional channels with currents and function similar to WT (Figure 1 and the Table). This finding is consistent with some12,13,24 but inconsistent with other25 reports that demonstrated that H558R expressed on the splice variant containing Q1077 demonstrated little to no whole-cell current. Therefore, future studies aimed at elucidating mechanisms of sodium channel rescue will have to include further data on both the Q1077-SCN5A and Q1077del-SCN5A.

The FRET data suggest that the domain 1 and 2 linker do not interact or have very weak interactions under normal conditions when 558 is a histidine in both proteins. Furthermore, the FRET studies potentially explain why WT-SCN5A channels do not rescue the R282H-SCN5A mutation because both mutant and WT channels express H558. Importantly, these data suggest that the degree of interaction between hNav1.5 subunits significantly increases when 1 protein encodes H558 and another encodes R558 (heterotypic proteins). However, the fact that WT-SCN5A (encoding H558)
cannot rescue R282H-H558R-SCN5A suggests that the polymorphism must be on the nonmutated protein. One speculated mechanism of this rescue is that the H558R polymorphism needs to be on the nonmutated protein to help restore folding of the mutated channel, which will then allow it to traffic to the cell membrane.

Because interaction between R282H-SCN5A and H558R-SCN5A appears to occur before protein trafficking to the membrane, this model of sodium channel α-subunit interaction also may help us better understand the mechanisms by which proteins fold and traffic within the endoplasmic reticulum (ER) and Golgi apparatus. Because the FRET measurements were made with 40-amino acid protein fragments, these data do not preclude heterotypic interaction of hNav1.5 in the cell membrane. Importantly, these results suggest that if hNav1.5 interaction occurs, it does so before protein trafficking to the membrane.

Previously, Biskup and colleagues suggested that the WT-SCN5A α-subunits do not interact on the basis of FRET measurements. In their studies, the CFP and YFP tags were added to the carboxyl terminus of hNav1.5. Because the base length dimension of the sodium channel is ~100 Å, it is possible that the distance between carboxyl termini is greater than the distance for FRET. Furthermore, others have demonstrated that the WT-SCN5A hNav1.5 α-subunit interacts with the β1-subunit in a 1:1 ratio in the ER before the complex is transported to the plasma membrane, which might further suggest that α-α-subunit interactions do not occur before or during protein transport between the ER, Golgi apparatus, or plasma membrane. Finally, studies of nonstationary fluctuation analysis fit to a binomial distribution for the number of open channels suggest that gating kinetics of single channels are independent of subunit interaction. More recently, Aldrich et al. suggested that WT-SCN5A subunits do not interact. However, further studies are needed to clarify this issue.

The mechanism by which the H558R polymorphism rescues the R282H mutation remains unknown. One potential explanation is that α-subunit interaction is not required for proper protein folding and trafficking, but it may be highly favorable. The low FRET values reported here also suggest that α-subunit interaction may be transient or facilitated by an intermediary protein. Because the FRET studies indicate that α-subunit interaction occurs before trafficking to the membrane, it is important to study whether this interaction occurs in the Golgi apparatus or ER. It could be that α-subunit interaction obscures an ER retention signal in a misfolded protein, as has been suggested with CFTR mutants. Another postulation is that α-α subunit interactions serve to stabilize both proteins for proper folding, thereby accomplishing multiple foldings in three-dimensional space. It is important to further note that it remains unclear how results obtained from an heterologous expression system translate into whole-heart electrophysiology. Significant research is still needed to fully understand how proteins fold and traffic.

Importantly, this study has many significant scientific implications. First, because of the incomplete penetrance nature of BrS, selective genotyping may not identify at-risk patients with 100% sensitivity without further consideration of complementary alleles. Second, heterotypic protein interaction may be a relatively unknown quality control mechanism. Furthermore, this study suggests that genetic polymorphisms may be a potential target for future therapies aimed at rescuing dysfunctional protein channels.

Sources of Funding
This study was supported by an American Heart Association Ohio Affiliate Beginning Grant-in-Aid (0465187B) (to Dr Deschênes) and the Michael Biliich Fellowship in Cardiac Pacing and Electrophysiology from the NASPE-Heart Rhythm Society (to Dr Poelzing).

Disclosures
None.

References
2. Brugada J, Brugada R, Brugada P. Right bundle-branch block and ST-segment elevation in leads V1 through V6; a marker for sudden death


**CLINICAL PERSPECTIVE**

Although the Brugada syndrome is an autosomal dominant disorder, it is characterized by incomplete penetrance, a phenomenon still incompletely understood. This study suggests that a dysfunctional protein produced by a mutation on one gene can be rescued by a complementary polymorphism on a separate allele. Although the in vitro results present a line of evidence consistent with the phenotype observed in the family depicted here, we cannot be sure that this is the exact mechanism of incomplete penetrance because these experiments are conducted in a heterologous expression system. However, this study demonstrates that a polymorphism on a second allele restores membrane trafficking of a specific mutant protein in vitro. Therefore, selective genotyping may not identify at-risk patients with 100% sensitivity without further consideration of complementary alleles. Furthermore, this study suggests that genetic polymorphisms may be a potential target for future therapies aimed at rescuing dysfunctional protein channels.
SCN5A Polymorphism Restores Trafficking of a Brugada Syndrome Mutation on a Separate Gene

Steven Poelzing, Cinzia Forleo, Melissa Samodell, Lynn Dudash, Sandro Sorrentino, Matteo Anaclerio, Rossella Troccoli, Massimo Iacoviello, Roberta Romito, Pietro Guida, Mohamed Chahine, Mariavittoria Pitzalis and Isabelle Deschênes

Circulation. 2006;114:368-376; originally published online July 24, 2006; doi: 10.1161/CIRCULATIONAHA.105.601294

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/114/5/368

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/