A Novel Strategy Using Cardiac Sodium Channel Polymorphic Fragments To Rescue Trafficking-Deficient SCN5A Mutations

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Background—Brugada syndrome (BrS) is associated with mutations in the cardiac sodium channel (Na,v1.5). We previously reported that the function of a trafficking-deficient BrS Na,v1.5 mutation, R282H, could be restored by coexpression with the sodium channel polymorphism H558R. Here, we tested the hypothesis that peptide fragments from Na,v1.5, spanning the H558R polymorphism, can be used to restore trafficking of trafficking-deficient BrS sodium channel mutations.

Methods and Results—Whole-cell patch clamping revealed that cotransfection in human embryonic kidney (HEK293) cells of the R282H channel with either the 40- or 20-amino acid cDNA fragments of Na,v1.5 containing the H558R polymorphism restored trafficking of this mutant channel. Fluorescence resonance energy transfer suggested that the trafficking-deficient R282H channel was misfolded, and this was corrected on coexpression with R558-containing peptides that restored trafficking of the R282H channel. Importantly, we also expressed the peptide spanning the H558R polymorphism with 8 additional BrS Na,v1.5 mutations with reduced currents and demonstrated that the peptide was able to restore significant sodium currents in 4 of them.

Conclusions—In the present study, we demonstrate that small peptides, spanning the H558R polymorphism, are sufficient to restore the trafficking defect of BrS-associated Na,v1.5 mutations. Our findings suggest that it might be possible to use short cDNA constructs as a novel strategy tailored to specific disease-causing mutants of BrS. (Circ Cardiovasc Genet. 2011;4:500-509.)

Key Words: arrhythmia ■ electrophysiology ■ gene therapy ■ ion channels ■ SCN5A protein

Brugada syndrome (BrS) is associated with an increased risk of sudden cardiac death resulting from episodes of polymorphic ventricular tachycardia.1,2 Mutations in the α-subunit of the cardiac sodium channel (Na,v1.5), encoded by the SCN5A gene, have been found in 15% to 30% of patients with BrS, currently representing the most common BrS genotype.3–5 To date, >100 SCN5A mutations have been linked to BrS.6 Most are missense mutations that lead to loss-of-function phenotypes by either decreasing sarcolemmal expression of Na,v1.5 channels,7,8 producing nonfunctional channels,9 or altering biophysical properties.10–13 Current treatment options for BrS include implantable cardioverter-defibrillator therapy and drug therapy mostly with isoproterenol.14 However, neither of these treatment options directly addresses the channel dysfunction that is the underlying source of BrS-associated arrhythmias.

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Failure of sarcolemmal expression has been identified as an important cellular mechanism of ion channel dysfunction in inherited arrhythmias, including BrS.7,8 For example, we have demonstrated that the BrS-causing mutation R282H produces a trafficking-deficient sodium channel.7 Interestingly, we also have identified a healthy proband despite carrying R282H. We demonstrated that this individual did not develop BrS because R282H trafficking was restored in the presence of the common sodium channel H558R polymorphism as a second allele.7 Here, we test a new strategy using peptide fragments of the Na,v1.5, spanning the H558R polymorphism, to restore trafficking and function of BrS mutations by directly aiding channel folding. This strategy is not without precedent because peptide fragments have already been shown to have potential for treating channelopathies. For example, the
most common mutation of the cystic fibrosis transmembrane conductance regulator $\Delta F508$-CFTR, which inhibits CFTR processing and trafficking, was rescued by coexpression of specific peptide or protein fragments derived from wild-type (WT) CFTR. In addition, it has been shown that trafficking of a human ether-a-go-go-related gene (hERG) mutant channel could be restored by peptide fragments spanning an endoplasmic reticulum (ER) retention signal exposed on the C-terminus of the channel protein.

We show that coexpression of BrS mutant channels with peptide fragments containing the H558R polymorphism similarly rescues protein trafficking and restores functional sodium channels to the cell surface. Finally, we used fluorescence resonance energy transfer (FRET) to analyze the mechanism of current restoration and show that peptide fragments containing the H558R polymorphism initiate the productive folding of originally processing-defective BrS mutants in the ER.

**Methods**

**Mutagenesis**

Nine BrS trafficking-deficient mutations located in the pore of domain 1 of the Na$_v$1.5 were studied. The R282H, G292S, V294M, K317N, L325R, G351V, D356N, R367H, and R376H mutations and the H558R polymorphism were created on the Nav1.5 background (PubMed Accession No. NM198056) expressed in the green fluorescent protein-internal ribosomal entry site vector (BD Biosciences Clontech; San Jose, CA) using the Stratagene QuikChange XL Site-Directed Mutagenesis Kit (Stratagene; La Jolla, CA).

**Creation of Channel Fragments**

For FRET experiments, channel fragments were created by amplifying small pieces of either the WT or the H558R polymorphism similar sodium channels to the cell surface. Finally, we used fluorescence resonance energy transfer (FRET) to analyze the mechanism of current restoration and show that peptide fragments containing the H558R polymorphism initiate the productive folding of originally processing-defective BrS mutants in the ER.

**Expression of Na$_v$1.5 and Fragments in HEK293 Cells**

Transient transfection of full-length Na$_v$1.5 cDNA and Na$_v$1.5 fragment cDNA into human embryonic kidney HEK293 cells was accomplished using the Polyfect (Qiagen; Valencia, CA) transfection kit according to the manufacturer’s protocol. When channels were expressed alone, $\frac{1}{2}$g of full-length Na$_v$1.5 cDNA was used. For cotransfections, $\frac{1}{2}$g of full-length Na$_v$1.5 cDNA and $\frac{1}{2}$g of Nav1.5 fragment cDNA were combined, leading to an excess of peptide in relation to the full-length channel. Assuming similar transfection levels for both full-length channels and the fragments, this would result in an estimated 90:1 or 45:1 molar ratio (peptide fragment:full-length sodium channel) for the 20-amino acid and 40-amino acid fragments, respectively, because of their differences in size. For direct application of the R558-containing peptide to the cell interior, a synthetic 20-amino acid peptide ([H]-NSTAGESESHR$^R$TSLLVPWPL-[OH]) was synthesized by Princeton BioMolecules (Langhorne, PA), which was added to the internal pipette solution at 10 $\mu$mol/L final concentration. Purity of the peptide was $\approx 98\%$.

**Biochemical Analysis of Na$_v$1.5 Channels**

HEK293 cells from similar confluent cultures were used to isolate proteins. Western blotting experiments were performed as previously described. To determine the expression level of the Na$_v$1.5 protein in total protein lysates, proteins were extracted from HEK293 cells transiently transfected with full-length Na$_v$1.5 cDNA or cotransfections of full-length Na$_v$1.5 cDNA with Na$_v$1.5 fragment cDNA. Nontransfected cells were used as the negative control. Equal amounts of protein extracts were separated on 3% to 8% Tris-acetate gel. Sodium channel proteins were detected using a rabbit polyclonal sodium channel antibody (Upstate).

Surface biotinylation experiments were performed according to the manufacturer’s protocol. Briefly, before cell lysis, surface pro-
teins were labeled at 4°C with 0.25 mg/mL Sulfo-NHS-SS-Biotin (Pierce). After cell lysis, biotinylated proteins were isolated through a NeutrAvidin Agarose resin column (Pierce), and after elution, western blot experiments were performed.

### Patch Clamp Experiments

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 (indicating the presence of the sodium channel) and cyan fluorescence (indicating the presence of the peptide) were considered successfully transfected. Low-resistance electrodes (<2 MΩ) were used, and a routine series resistance compensation of an Axopatch 200A was performed to values of >80% to minimize voltage clamp errors. The uncompensated $R_{series}$ was therefore <2 MΩ. Voltage clamp command pulses were generated by a microcomputer using PCLAMP software version 9.02 (Axon Instruments; Foster City, CA). 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, MgCl₂ 1, CaCl₂ 2, HEPES 10, and CsCl 10 adjusted to pH 7.4. Experiments were performed at room temperature (22°C–23°C). Whole-cell sodium current densities, cell capacitance, current-voltage relationship, conductance, time course of recovery from inactivation, steady-state inactivation, and persistent current were measured as previously described.

### Constructs for FRET

The FRET construct was created using a transposon approach, which randomly inserts enhanced yellow fluorescent protein (EYFP) from the transposon construct into the Nav1.5 construct. The p(AmpR)R6Kyori transposon (Epicentre Biotechnologies; Madison, WI) was altered to contain EYFP upstream of the Amp (ampicillin) gene. Through mutagenesis, restriction sites were created on each end of the Amp gene to facilitate removal. The newly formed EYFP gene. Through mutagenesis, restriction sites were created on each end of the Amp gene to facilitate removal. The newly formed EYFP gene containing the polymorphism WT and 2B) to 75% of the WT level. However, when WT channels were coexpressed with the 40-amino acid fragment containing the polymorphism WT+R558-40aa, the current density increased to 30% of the WT level. Furthermore, coexpression of a 20-amino acid fragment containing the polymorphism WT+R558-20aa further increased current density (Figure 2A and 2B) to 75% of the WT level. However, when WT channels were coexpressed with the 40-amino acid fragment containing the polymorphism WT+R558-40aa, the current density was not significantly different from WT channels alone (−474.1±125.9 versus −337.7±105.8 pA/pF, P>0.05). These results indicate that a small R558-containing peptide is able to specifically restore trafficking of the mutant channel.

To ascertain that the polymorphism was necessary for rescue, the mutant channel was coexpressed with the corresponding 20-amino acid fragment of the WT channel R282H+H558-20aa (Figure 2A and 2B). In this condition, restoration of current was not observed, indicating that the polymorphism is required to restore defective trafficking of the R282H mutant channel. To rule out any acute effects of the peptide, the 20-amino acid R558-containing peptide was synthesized and applied in the intracellular solution during whole-cell recordings. Acute expression of synthetic peptide did not alter peak current density of WT (−478.3±104.4 pA/pF, n=6) and did not restore function of the R282H mutant channel (−23.8±4.2 pA/pF, n=7).

### Biophysical Properties of Rescued Currents

Having shown that R558-containing peptides fused to CFP were capable of restoring trafficking of the R282H mutant...
channel, we next compared the biophysical properties of rescued currents to WT currents. The current-voltage relationship, activation curve, voltage dependence of steady-state inactivation, recovery from inactivation, and time constant of inactivation were measured for WT and rescued R282H currents. We found that the normalized current-voltage relationships (Figure 2C) were not different between rescued and WT currents. The activation curve (Figure 3A) for rescued currents was also similar to WT. However, rescued R282H currents differed from WT currents in steady-state inactivation, which was shifted to more depolarized voltages, and recovery from inactivation was faster (Figure 3B and 3C, Table 1). The time course of inactivation (Figure 3D) was slower for channels rescued with the peptides but only reached significance for channels treated with the smaller 20-amino acid peptide. Because the channel appeared to have impaired inactivation, we also measured the level of tetrodotoxin-sensitive persistent current at the end of 300-ms depolarizing pulse for the R282H currents rescued by the R558-40aa peptide.
These experiments demonstrated a significant increase in the level of persistent current (R282H, 0.37 ± 0.08% [n=7]; WT, 0.15 ± 0.07% [n=5]). Importantly, none of these differences were observed when WT channels were treated with the R558-containing peptide WT/R558-40aa (Figure 3). Taken together, the biophysical data suggest that the rescued R282H channels express a gain-of-function phenotype dominated by impaired inactivation and that the fragments do not affect biophysical properties of WT channels.

Peptides Containing the H558R Polymorphism Increase Na1.5 Protein Level in the Sarcolemma

Next, we sought to determine whether the restoration of the R282H mutation by the R558-containing peptide was due to alteration of Na1.5 protein expression or correction of defective channel trafficking. First, we transiently expressed the R282H mutation with or without the 20-amino acid fragment containing the polymorphism (R558-20aa). Then, we evaluated the expression level of the Na1.5 proteins by western blots by first measuring whole-cell lysate and noticed that the expression level of R282H was similar to that of WT Na1.5 proteins (Figure 4A). Figure 4A shows that the presence of the fragment containing the H558R polymorphism R558-20aa did not alter the total expression level of the R282H proteins in whole-cell lysate. In fact, sodium channel protein levels were similar for WT and R282H (with or without the R558-containing peptide). Because we observed an increase in peak current density for the R282H mutant in the presence of R558-containing peptides (Figure 2A and 2B), we performed surface biotinylation experiments to investigate the absence or presence of the R282H mutant in the cell membrane with or without the R558-containing peptide. Figure 4B and 4C confirm the absence of the R282H mutant in the cell membrane, as expected for a trafficking-deficient mutant. Importantly though, in the presence of the R558-containing peptide, the R282H mutant sodium channel is inserted into the cell membrane, in line with current recordings.

R282H Affects Channel Conformation

To function properly, newly synthesized ion channels need to assemble and fold correctly in the ER and traffic properly to

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<th>Table 1. Electrophysiological Properties for WT and BrS Mutant hNa1.5 Channels in the Presence or Absence of the Peptide Fragments</th>
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<tr>
<td>------------------------------------------</td>
</tr>
<tr>
<td>WT</td>
</tr>
<tr>
<td>WT + R558-40aa</td>
</tr>
<tr>
<td>R282H</td>
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<tr>
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Data are presented as mean ± SEM. BrS indicates Brugada syndrome; hNa1.5, human cardiac sodium channel; na, not available because of the small currents; WT, wild type.

*P<0.01 compared with WT.
†P<0.01 compared with mutant alone.
their respective membrane subdomain.\textsuperscript{21} We have demonstrated previously that the absence of current observed with the R282H mutation is due to a trafficking defect.\textsuperscript{7} A common cause of trafficking-deficient channels is misfolding and retention by the quality control machinery in the ER.\textsuperscript{22–24} Thus, a possible mechanism by which the R558-containing peptide may rescue the R282H mutant is by restoring proper folding of the mutated channel to an exportable conformation. FRET was used to examine folding of the trafficking-deficient mutant channel R282H in the presence and absence of the R558-containing peptide. To accomplish this, a sodium channel construct containing a pair of fluorescent proteins capable of transferring energy from one to the other was created.

The Na\textsubscript{v}1.5 used for these experiments, EYFP-1022, had ECFP fused to the C-terminus of the channel and EYFP inserted into the domain II-III linker at amino acid position 1022 (Figure 5A). This construct produced functional channels (Figure 5B). EYFP-1022 was associated with a FRETc value of 0.27±0.01 (Figure 5C). This value was significantly greater than the negative control (0.16±0.01, \textit{P}<0.001). When the R282H mutation was created on EYFP-1022 (EYFP-1022/R282H), the FRETc value decreased significantly to 0.18±0.01 but was still significantly different from negative control (\textit{P}=0.02). The FRET decrease indicated that the 3D conformation of the channel has changed so that the EYFP and ECFP were now farther apart. This is likely because the R282H mutant channel is improperly folded. Interestingly, when fragments containing the polymorphism were coexpressed with the EYFP-1022/R282H construct, FRETc was restored to values similar to nonmutated EYFP-1022 (\textit{P}>0.05) (Figure 5C), which suggests that the mutated channel EYFP-1022/R282H has now obtained a conformation similar to that of the functional EYFP-1022 channel and likely explains the ability of the mutant channel to traffic in the presence of R558-containing peptide. Similarly, when the EYFP-1022/R282H construct was expressed in the presence of the drug mexiletine, which is known to restore trafficking of the R282H mutant, FRETc values also were restored to the level of the nonmutated EYFP-1022 (data not shown), supporting the hypothesis that the mutant channel, either in the presence of the drug or with the R558-containing peptide, is now folding properly to allow the channel to traffic to the cell membrane, forming functional channels.

To exclude nonspecific effects of the peptide, nonmutated EYFP-1022 channels were coexpressed with R558-containing peptide. No FRETc increase was observed in these cells (Figure 5C), confirming that the increase in FRETc seen when EYFP-1022/R282H was coexpressed with the R558-containing peptide is specific. To further verify that the polymorphism was necessary for rescuing the improperly folded EYFP-1022/R282H channel, the EYFP-1022/R282H channel was coexpressed with the corresponding 40- or 20-amino acid fragment of the WT channel. In this experiment, restoration of FRETc values were not observed (Figure 5C), indicating that the polymorphism is required to promote the proper folding of the R282H channel.

Thus, these data suggest that restoration of folding in the ER will lead to protein trafficking to the sarcolemma, which will result in the increase in peak current density observed (Figure 2A and 2B). To separate folding effects in the ER from forward trafficking to the cell surface membrane, we used the fungal metabolite BFA, which prevents anterograde transport from the ER to the Golgi apparatus. BFA treatment is believed to lead to a rapid accumulation of proteins within the ER after collapse of the Golgi apparatus.\textsuperscript{25,26} Consequently, incubation of cells coexpressing the R282H mutation and the R558-containing
peptide (R282H + R558-20aa, n = 6) together with BFA did not generate functional sodium current. In marked contrast, this blocking of forward trafficking with 5-µg/mL BFA applied for 24 hours did not alter FRETc values (P > 0.05) (Figure 5C). These results indicate that conformational changes induced by EYFP-1022/R282H take place in the ER and not en route to the cell surface. Taken together, the FRET data suggest that the peptide containing the polymorphism rescues the R282H mutant by restoring proper folding of the mutated channel in the ER.

### Discussion

BrS is associated with mutations in Na,1,5 that decrease whole-cell sodium currents, often by reducing channel expression at the cell surface. This lack of whole-cell sodium current is believed to cause an increased risk of sudden cardiac death in patients with BrS.27,28 In the present study, we showed that small peptides, spanning the H558R polymorphism, are sufficient to restore the trafficking defect of a subset of BrS-associated Na,1,5 mutations. The central hypothesis of this work was first based on our previous study, which provided us with enough supporting evidence of the rescue of the R282H mutant by the H558R polymorphism (both clinical profile and cellular electrophysiology). 

However, we further hypothesized that this new strategy using peptide fragments of Na,1,5, spanning the H558R polymorphism, could be used to restore the trafficking and function of other BrS mutations in which reduced sodium current densities also are observed. Nine BrS trafficking-deficient mutations located in the pore of domain 1 of the Na,1,5 were studied. The peptide containing the H558R polymorphism was able to increase current densities in 5 of these mutants. To our knowledge, this study is the first to show that short fragments containing H558R polymorphism can correct a defective trafficking phenotype in BrS-associated Na,1,5 mutations. The FRET experiments suggest that the R558-containing peptides may act as “chemical chaperones” that promote proper protein folding and thereby facilitate the exit of the rescued mutant ion channel from the ER.

It is commonly believed that channels that fail to traffic to the cellular membrane are misfolded, but very little work has been done to test this notion. Therefore, we used a FRET-based approach to study folding of the channel. To the best of our knowledge, this FRET approach has not been used to study folding of sodium channels. When R282H was introduced in a functional channel construct containing CFP and YFP, the folding of the channel became altered, as shown by the decrease in FRETc signal and confirmed by the severe reduction of whole-cell sodium currents produced by the R282H mutation (Figure

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Data are presented as mean ± SEM. The following mutants did not produce currents when coexpressed with the R558-20aa peptide: L325R, G351V, D356N, and R367H. Abbreviations as in Table 1.

*P < 0.01 compared with WT.
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The Peptide Fragment Containing the H558R Polymorphism Can Restore Currents for Multiple BrS Mutants

To expand on our observations with R282H, we tested the following 8 additional sodium channel BrS mutations located in the same region as R282H (pore domain 1): G292S, V294M, K317N, L325R, G351V, D356N, R367H, and R376H (Figure 1). The mutations were taken from the Inherited Arrhythmia Database Web site (www.fs.bumc.edu/cardiac). Similarly to R282H, all mutants expressed alone resulted in significantly decreased sodium currents compared with WT (Table 2). In fact, the K317N, L325R, G351V, D356N, R367H, and R376H resulted in nonmeasurable currents. For the G292S and V294M mutants, small and significantly reduced currents compared with WT were measured. Importantly, when these mutants were coexpressed with the R558-20aa peptide, the current density was increased for G292S, V294M, K317N, and R376H (Table 2). The biophysical properties of these rescued currents were similar to WT channels, except for K317N, which displayed a shift toward depolarized voltages for steady-state inactivation (Table 2). However, even in the presence of the R558-containing peptide, there were still no measurable currents for L325R, G351V, D356N, and R367H (Table 2). Thus, we now show that out of 9 BrS-associated Na,1,5 mutations tested, the peptide was able to restore large sodium currents in 5 of them, confirming that this mechanism/strategy is not unique to the R282H mutation.

### Table 2. Electrophysiological Properties for BrS Mutant hNa,1,5 Channels in the Presence or Absence of the R558-20aa Peptide

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5). Previous studies have shown that this mutant channel is retained in the ER, and it appears that altered folding may be the cause of ER retention. In the presence of either of the peptides containing the H558R polymorphism (40aa and 20aa), folding of the R282H mutant channel, as indicated by the restored FRET value, was corrected. These data suggest that in the presence of the R558-containing peptide, the mutant channel is able to fold properly. Furthermore, blocking forward protein trafficking by BFA did not alter FRET values. These data confirm that the R558-containing peptides specifically rescue the R282H mutant by restoring proper folding of the mutated channel in the ER, indicating that the mechanism by which the polymorphism rescues the mutant channel either directly assists in channel folding or allows another protein to interact with the channel to correct folding.

A growing body of literature suggests that trafficking defect of mutant channels can be rescued by fragments of polypeptides. Fragments of the hERG and CFTR channel have shown to be restored to mutant channels. Kupershmidt et al showed that a 100-amino acid peptide was able to restore trafficking to an hERG mutation in which the C-terminal region was truncated. When a peptide containing the ER signal RXR was coexpressed with the mutant channel, the peptide acted as a decoy, allowing the mutant channel protein to bypass the ER quality control system. Moreover, Owsianik et al proposed a similar mechanism for the ability of peptides to rescue the ΔF508-CFTR mutation. However, after observing a similar rescue of the same channel, Cormet-Boyaka et al proposed that several different mechanisms may contribute to the fragments’ ability to help the mutant CFTR channel process, including that the fragments could be providing ER exit codes or burying areas on the CFTR channel that would lead to channel aggregation and destruction. However, none of these mechanisms seem to be occurring with the present sodium channel fragments. Three RXR endoplasmic retention signals have been identified in the domain I-II linker of Na1.5, the region from which our fragments originate, but none are present in the fragments. Thus, it seems unlikely that they are acting as decoys. The FRET data also suggest that the fragments are involved in folding rather than in providing or hiding ER signals.

The rescue of sodium channel mutations with mexiletine has been well documented. The theory behind these rescues is that the drug, which is a Na1.5 blocker, stabilizes the pore of the channel, allowing for trafficking. It is possible that the R558-containing peptides work similarly in that they may be able to stabilize part of the channel for proper folding in the ER and thus restore trafficking. However, it appears unlikely that the fragments are acting as channel blockers. First, we did not observe a reduction of current density when WT channels were coexpressed with either the 40-amino acid fragment or in the presence of the synthesized 20-amino acid R558-containing peptide. If the fragment was acting as a blocker, we would expect to see some decrease in whole-cell current density in both transient transfection and acute effect experiments. Additionally, we know that the whole polymorphic channel can rescue the mutant channel, and therefore, it is unlikely that the polymorphic channel is blocking the mutant channel. Moreover, R558-containing peptide did not alter the expression level of the R282H proteins (Figure 4A). Thus, we suspect that the mechanism of rescue with the R558-containing peptides differs from the one of sodium channel blockers. However, previous work in hERG has shown that channel blocking is not necessarily required for the rescue of trafficking-deficient channels to occur. Thus, it is possible for the mechanism of rescue of such drugs and of the peptide to be similar and yet different from the blocking effect.

When trafficking is restored to the mutant R282H and K317N channels, changes in biophysical properties are uncovered, suggesting the complexity of the disease. This finding is also consistent with a growing body of literature that suggests that some SCN5A mutations can cause both long-QT and BrS phenotypes in members of 1 family or long QT in 1 family and BrS in another. Pfahnl et al recently described a similar phenomenon involving another trafficking-deficient BrS mutation, T353I, rescued by mexiletine. Interestingly, overnight exposure to 0.1 mmol/L mexiletine increased T353I channel trafficking to the membrane to near-normal levels. However, this mutant channel also showed a significant increase in late current by 1.6%, a finding seen with long-QT mutations.

The destabilization of inactivation observed in rescued R282H mutant channels is consistent with the biophysical changes caused by mutations associated with long-QT syndrome type 3. It is plausible that the presence of a small late sodium current may be masked because of the inefficient channel trafficking, giving rise to the concern that protecting a patient from BrS may cause another type of arrhythmia. However, the initial patient identified by Poelzing et al carries both the polymorphism and mutation and is healthy, displaying symptoms of neither BrS nor long-QT syndrome. The possible explanation for the discrepancy in the alteration of inactivation kinetics after the R558-containing peptide treatment might be due to the difference in sodium channel population expressed in our heterologous expression system. In the present experiment, only the R282H channel is expressed, whereas in the previous report, both the H558R polymorphic channel and the R282H channel were expressed. Therefore, in the latter experiment, the whole-cell currents measured are the summation of the currents flowing through both the H558R polymorphic channel and the rescued R282H channel. Thus, we speculate that the kinetics of the currents generated from the H558R polymorphic channel might conceal the alteration in fast inactivation of the rescued R282H mutant channel.

We have shown that small peptides, spanning the H558R polymorphism, are sufficient to restore trafficking defects of some BrS-associated Na1.5 mutations. Because of their ability to rescue a mutant channel in vitro, we reasoned that it may be possible to use this R558-containing peptide as an alternative strategy to rescue the trafficking defect of mutant Na1.5 in vivo. We acknowledge the presence of
limitations in our study. First, this rescuing strategy works on a subset of mutations, and we are not aware yet of the exact mechanism that underlies this discrepancy between mutations. Further studies are needed to test whether this rescue will occur in vivo. Importantly, although a future clinical application will require several further investigations, the present study remains the first to present a new alternative strategy for treating the trafficking defects of BrS-associated sodium channels that addresses the underlying cause of the disease by restoring the function of the mutant channels. Additionally, this new approach has the advantage of using only a small fragment of the target gene, which should reduce the problems usually seen with gene therapy when an entire gene is transferred. Therefore, polymorphic-containing peptides might represent a promising option to rescue trafficking-deficient channels.

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**Disclosures**

None.

**References**


Clinical Perspective

Brugada syndrome (BrS) is an inherited primary electric cardiac disorder. It is characterized by ST-segment elevation in the right precordial leads (V1–V3) and increased susceptibility to sudden cardiac death because of episodes of polymorphic ventricular tachycardia. The genetic abnormalities that cause BrS have been linked to mutations in the SCN5A gene, which encodes for the pore-forming α-subunit of the cardiac sodium channel (Na_1.5). A trafficking defect of mutant Na_1.5 has been identified as an important cellular mechanism underlying a loss-of-function of Na_1.5 in BrS. Current treatment options for BrS include implantable cardioverter-defibrillator therapy and drug therapy mostly with isoproterenol. However, neither of these treatment options directly addresses the channel dysfunction at the underlying source of BrS-associated arrhythmias. We previously reported that the function of a trafficking-deficient BrS Na_1.5 mutation, R282H, could be restored by coexpression with Na_1.5 containing the H558R polymorphism. In the present study, we demonstrate that small peptides, spanning the H558R polymorphism, are sufficient to restore the trafficking defect of BrS-associated Na_1.5 mutations. Therefore, peptides containing the H558R polymorphism might represent a promising option to rescue trafficking-deficient BrS mutations. In addition, our findings suggest that it might be possible to use short cDNA constructs as a novel strategy tailored to specific disease-causing mutations associated with BrS.
A Novel Strategy Using Cardiac Sodium Channel Polymorphic Fragments To Rescue Trafficking-Deficient SCN5A Mutations
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