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Characterization and Mechanisms of Action of Novel $\text{Na}_v1.5$ Channel Mutations Associated With Brugada Syndrome

Kirstine Calloe, MSc, PhD*; Marwan M. Refaat, MD*; Soren Grubb, MSc; Julianne Wojciak, MSc; Joan Campagna, CCMA; Nancy Mutsaers Thomsen, BSc; Robert L. Nussbaum, MD; Melvin M. Scheinman, MD; Nicole Schmitt, MSc, PhD

Background—Brugada syndrome is a heterogeneous heart rhythm disorder characterized by an atypical right bundle block pattern with ST-segment elevation and T-wave inversion in the right precordial leads. Loss-of-function mutations in *SCN5A* encoding the cardiac sodium channel $\text{Na}_v1.5$ are associated with Brugada syndrome. We found novel mutations in *SCN5A* in 2 different families diagnosed with Brugada syndrome and investigated how those affected $\text{Na}_v1.5$ channel function.

Methods and Results—We performed genetic testing of the probands' genomic DNA. After site-directed mutagenesis and transfection, whole-cell currents were recorded for $\text{Na}_v1.5$ wild type and mutants heterologously expressed in Chinese hamster ovary-K1 cells. Proband 1 had two novel $\text{Na}_v1.5$ mutations: $\text{Na}_v1.5$ -R811H and $\text{Na}_v1.5$ -R620H. The $\text{Na}_v1.5$ -R811H mutation showed a significant loss of function in peak Na^+ current density and alteration of biophysical kinetic parameters (inactivation and recovery from inactivation), whereas $\text{Na}_v1.5$ -R620H had no significant effect on the current. Proband 2 had a novel $\text{Na}_v1.5$ -S1218I mutation. $\text{Na}_v1.5$ -S1218I had complete loss of function, and 1:1 expression of $\text{Na}_v1.5$ -wild type and $\text{Na}_v1.5$ -S1218I mimicking the heterozygous state revealed a 50% reduction in current compared with wild type, suggesting a functional haploinsufficiency in the patient.

Conclusions— $\text{Na}_v1.5$ -S1218I and R811H are novel loss-of-function mutations in the *SCN5A* gene causing Brugada syndrome. (*Circ Arrhythm Electrophysiol.* 2013;6:177-184.)

Key Words: arrhythmia ■ Brugada syndrome ■ electrophysiology ■ mutation ■ *SCN5A* ■ sodium

Brugada syndrome (BrS) is a rare clinical condition characterized by atypical right bundle branch block and elevated ST segments in right precordial leads in the absence of structural heart disease.¹ Diagnosis is sometimes difficult because the same pattern can be seen in patients with other cardiac diseases, such as arrhythmogenic right ventricular cardiomyopathy, myocardial infarction, and others.² Hence, additional clinical features, including documented ventricular fibrillation, polymorphic ventricular tachycardia, a family history of sudden cardiac death at <45 years of age, coved-type ECGs in family members, syncope, nocturnal agonal respiration, or febrile-initiated triggers, aid in the diagnosis.³⁻⁷

Clinical Perspective on p 184

BrS is inherited as an autosomal-dominant trait, with incomplete penetrance.⁸ To date, mutations in 9 genes encoding ion channel subunits have been associated with BrS (*SCN5A*, *SCN1B*, *SCN3B*, *CACNA1C*, *CACNB2B*, *CACNA2D1*, *KCNJ8*, *KCND3*, and *KCNE3*).⁹⁻¹² In addition, gene products affecting cardiac ion

currents have been linked to or suggested as susceptibility genes (*GPDL*, *MOGI*).^{13,14} The majority of these mutations have been reported in the *SCN5A* gene encoding the cardiac sodium channel $\text{Na}_v1.5$ which is the molecular basis of I_{Na} , the current underlying the depolarization (phase 0) of the cardiac action potential.¹⁵

In this study, we identified novel mutations in *SCN5A* in 2 independent families with BrS and investigated how these mutations affected $\text{Na}_v1.5$ channel function.

Methods

Study Subjects

The study conforms to the principles outlined in the Declaration of Helsinki and was approved by the Institutional Review Board of the University of California San Francisco. All included patients gave written informed consent.

Mutation Screening

Genetic screening of the probands and family members was performed at Gene Dx (Gaithersburg, MD) analyzing the genes *SCN5A*, *CACNA1C*, *CACNB2*, *GPDL*, *KCNE3*, *SCN1B*, and

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SCN3B. The control group consisted of 400 healthy subjects of white and black ancestry.

Mutagenesis

The cDNA encoding hNa_v1.5 refers to isoform 2 (GenBank Acc. No. NM_000335) and is also known as hH1c,¹⁶ that is, it lacks Q1077. The nomenclature of the variants refers according to conventions to isoform 1 (NM_198056).¹⁷ The point mutations R620H (c.1859G>A), R811H (c.2432G>A), and S1218I (c.3653G>T) in Na_v1.5 were introduced using mutated oligonucleotide extension (PfuTurbo Polymerase; Stratagene, La Jolla, CA) from the plasmid template hNa_v1.5 in pcDNA3.1, digested with *DpnI* (Fermentas, St. Leon-Roth, Germany) and transformed into *Escherichia coli* XL1 Blue cells. All plasmids were verified by complete DNA sequencing of the cDNA insert (Macrogen Inc, Seoul, Republic of Korea).

Cell Culture and Transfections

Chinese hamster ovary-K1 cells were kept in DMEM (University of Copenhagen, Denmark) supplemented with 10% FCS (GIBCO, Invitrogen, Carlsbad, CA) and 40 mg/L L-proline at 37°C in 5% CO₂. Cells were transfected with 2 µg hNa_v1.5; in coexpression experiments, wild type (WT) and mutants were transfected in a 1:1 molar ratio. Transfections were performed using Lipofectamine and Plus reagent (GIBCO, Invitrogen) according to the manufacturer’s instructions. Enhanced green fluorescent protein (0.2 µg) was included to identify transfected cells.

Electrophysiology

Currents were recorded 2 to 3 days after transfection using a MultiClamp 700B amplifier and MultiClamp Commander (Axon

Instruments, Molecular Devices, Biberach an der Riss, Germany). Cells were superfused with a solution containing the following (in mmol/L): 130 NaCl, 5 KCl, 1.8 CaCl₂, 1.0 MgCl₂, 2.8 Na-acetate, 10 HEPES, and 10 glucose, pH=7.3 with NaOH. Patch pipettes were fabricated from borosilicate glass capillaries (Module Ohm, Herlev, Denmark) and had resistances between 1.5 and 2 MΩ when filled with pipette solution (in mmol/L): 10 KCl, 105 CsF, 10 NaCl, 10 HEPES, 10 EGTA, and 5 TEA-Cl, pH=7.2 with CsOH. Electronic compensation of series resistance to 70% to 85% was applied to minimize voltage errors. All analog signals were acquired at 10 to 50 kHz, filtered at 6 kHz, digitized with a Digidata 1440 converter (Axon Instruments), and stored using pClamp10 software (Axon Instruments). All recordings were made at room temperature (20°C –22°C). Data were analyzed using pClamp10 software (Axon Instruments) and Prism (GraphPad Software, La Jolla, CA).

Data Analysis

Mean±SEM values are shown; statistical significance (*P*<0.05) was evaluated by 1-way ANOVA, followed by Student–Newman–Keuls post-test or by an unpaired Student *t* test. Because of the relatively low sample size, significance was confirmed by a Mann–Whitney rank-sum test.

Results

Clinical Characterization

Proband 1 (Figure 1): The patient is a 44-year-old white man of Italian ancestry with history of recurrent episodes of syncope. He was first admitted in February 2010 after an episode

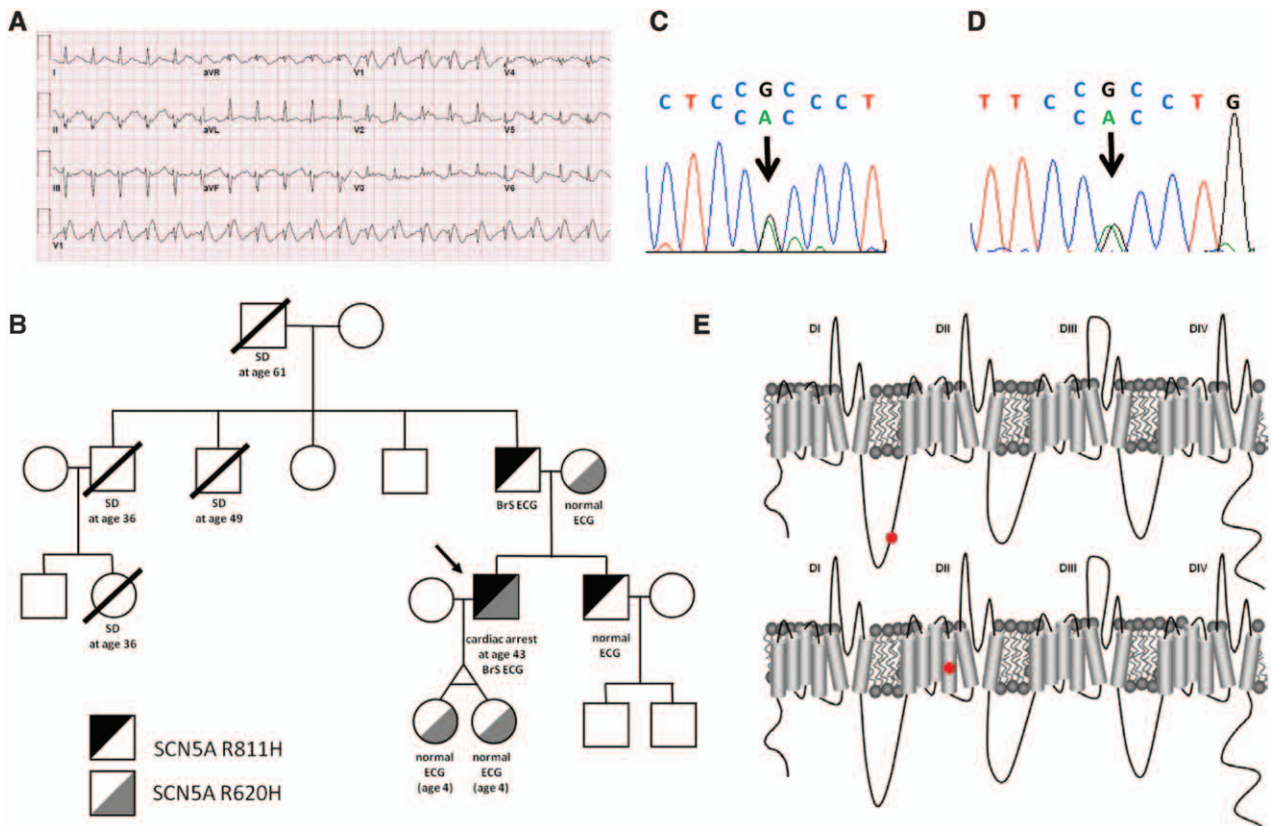


Figure 1. Clinical and genetic information of proband 1. **A**, ECG of proband. **B**, Pedigree for the family is shown (circles=women; squares=men) with phenotypic and genotypic information where available. The half-filled color in black indicates R811H mutation carrier, whereas the half-filled color in gray indicates R620H mutation carrier. The **arrow** marks the patient diagnosed with Brugada syndrome (proband). **C**, Mutation c.1859G>A resulting in p.Arg620His. **D**, Mutation c.2432G>A resulting in p.Arg811His. **E**, Topological position of the identified mutations shown in red (**top**, R620H; **bottom**, R811H). DI–DIV denotes the 4 homologous domains of the Na_v1.5 channel.

of rapid ventricular tachycardia requiring direct-current shock. The ECG was characteristic of the Brugada pattern with a PR interval measuring 222 ms, QRS interval measuring 158 ms, and QT_c interval measuring 416 ms. He had an implantable cardioverter-defibrillator. He was readmitted 6 months later with a febrile illness attended by recurrent ventricular tachycardia and had a total of 2 shocks en route to our emergency facility. He is currently treated with quinidine and theophylline and remains stable.

The family history revealed a history of sudden death of one paternal uncle at 49 years of age and another at 36 years of age, together with the sudden death of a female cousin at 36 years of age. The proband's father had an ECG showing the type I Brugada pattern and was asymptomatic. The proband's mother had a normal ECG and no family history suggestive of BrS. The proband's brother was asymptomatic and had a normal ECG (PR interval, 186 ms; QRS interval, 98 ms; and QT_c interval, 431 ms).

Proband 2 (Figure 2): The patient is a 33-year-old white man (Swedish and German ancestry) with history of recurrent syncope that occurred both at rest and with exercise (yoga class of moderate intensity). He had a syncopal episode in December 2009 and underwent an electrophysiology study at another hospital and had inducible atrial flutter that was successfully ablated. At least 1 ECG showed a typical Brugada pattern with a PR interval measuring 297 ms, QRS interval measuring 125 ms, and QT_c interval measuring 400 ms. A

repeat electrophysiology study at our medical center showed a His-ventricular interval of 79 ms and no inducible ventricular tachycardia. He had an implantable cardioverter-defibrillator. Family history revealed sudden death of his maternal grandmother (who had the Brugada pattern on ECG with a PR interval measuring 204 ms, QRS interval measuring 164 ms, and QT_c interval measuring 455 ms) and a maternal great uncle at 60 years of age and history of repeated syncope in a maternal great uncle's brother. The proband's father had an ECG that shows a normal sinus rhythm and left anterior fascicular block (PR interval, 182 ms; QRS interval, 120 ms; and QT_c interval, 463 ms). The proband's mother had the Brugada pattern with a PR interval measuring 218 ms, QRS interval measuring 102 ms, and QT_c interval measuring 434 ms. His brother had the coved-down Brugada pattern (PR interval, 246 ms; QRS interval, 122 ms; and QT_c interval, 389 ms), as well as an abnormal procainamide challenge, and underwent implantable cardioverter-defibrillator implantation.

Mutation Screening

Direct DNA sequencing of the probands' DNA revealed novel mutations. Proband 1 is compound heterozygous for 2 novel variants, c.1859G>A and c.2432G>A, leading to amino acid changes R620H and R811H, respectively. The variant R811H, which resides in the fourth transmembrane segment of DII was inherited from the father, the side of his family where the other BrS relatives are located, whereas

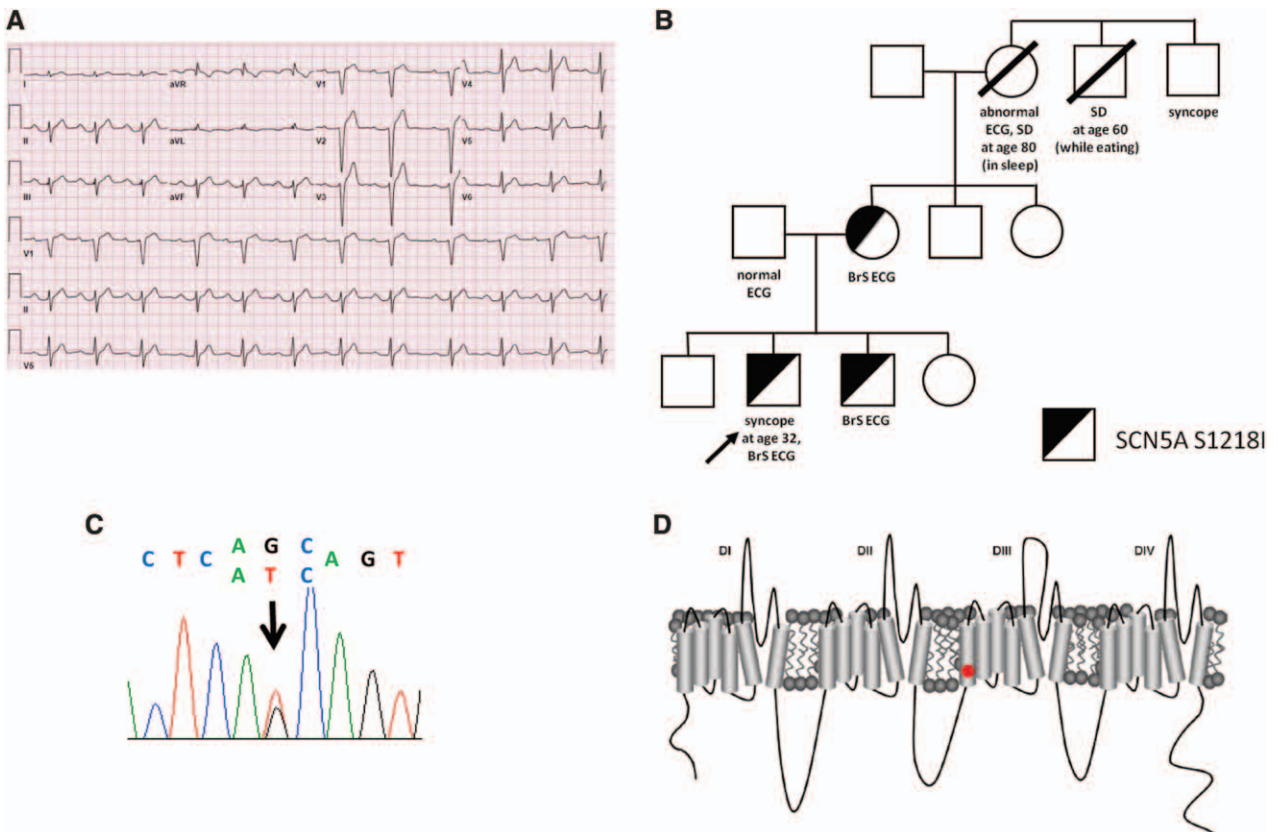


Figure 2. Clinical and genetic information of proband 2. **A**, ECG of proband. **B**, Pedigree for the family is shown with phenotypic and genotypic information, symbols as in Figure 1. Half-filled color in black indicates S1218I mutation carrier. **C**, Mutation c.3653G>T resulting in p.Ser1218Ile. **D**, Topological position of the identified mutation S1218I shown in red. DI–DIV denotes the 4 homologous domains of the Na_v1.5 channel.

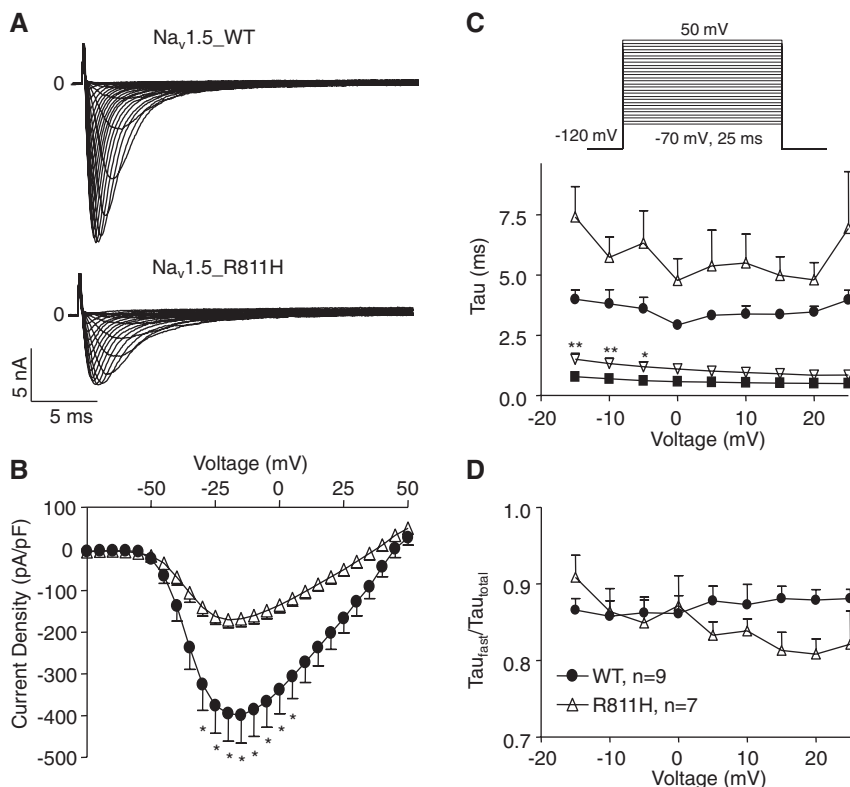


Figure 3. Electrophysiological characterization of R811H. **A**, Representative whole-cell current recordings for $\text{Na}_v1.5$ wild type (WT; $n=9$) and R811H ($n=7$) transiently expressed in Chinese hamster ovary-K1 cells. Currents were activated by the depicted voltage-clamp protocol. **B**, Peak current density plotted as a function of voltage, demonstrating a reduction in current for R811H. **C**, Double-exponential functions were fitted to the current decay, and the resulting time constants (τ_{slow} and τ_{fast}) are shown as a function of voltage. **D**, The relative weight of the pre-exponential factor for τ_{fast} .

the mother carried R620H, which is located in the D1–DII linker region (Figure 1C–1E). His brother carries the R811H variant but not the R620H variant. His twin daughters carry the R620H variant.

Proband 2 is heterozygous for a novel *SCN5A* mutation, c.3653G>T, leading to an amino acid substitution S1218I. His mother and brother also carried the S1218I mutation. No other variants likely to contribute to disease were found. The mutation resides in the transmembrane segment S1 of domain III (Figure 2C and 2D)

All amino residues found to be mutated in this study were highly conserved among mammalian species. The novel variants were absent in the control group of 400 healthy subjects of white and black ancestry.

Electrophysiological Characterization of $\text{Na}_v1.5$ -R811H and R620H

$\text{Na}_v1.5$ -WT or $\text{Na}_v1.5$ -R811H was expressed in Chinese hamster ovary-K1 cells. Both WT and R811H channels exhibited a fast activating and inactivating current (Figure 3A). However, R811H had a significantly reduced peak current density (Figure 3B). Double exponential fits to the current decay showed that τ_{fast} was significantly slower for R811H (Figure 3C). The relative weight of the pre-exponential factors for τ_{fast} was calculated (Figure 3D), showing that the relative contribution of the fast component was not significantly different between R811H and WT.

Steady-state activation curves were calculated by normalizing the currents shown in Figure 3 to $(V_m - E_K)$. The half-maximal voltage $V_{1/2}$ of steady-state activation was not affected by the R811H mutation, with $V_{1/2} = -32.7 \pm 0.2$ mV ($n=7$) for WT and $V_{1/2} = -33.3 \pm 0.4$ mV ($n=7$) for R811H (Figure 4). Steady-state inactivation was addressed by

a prepulse-pulse protocol, and representative traces are depicted in Figure 4A. For WT, the $V_{1/2}$ of inactivation was -69.5 ± 0.2 mV ($n=7$) and for R811H it significantly shifted to $V_{1/2} = -77.9 \pm 0.2$ mV ($n=7$; $P=0.049$). This negative shift in $V_{1/2}$ of inactivation would further contribute to a loss-of-function phenotype for R811H.

Recovery from inactivation of WT and R811H was addressed by a 2-pulse protocol using increasing interpulse intervals at -120 mV (Figure 5). At both potentials, the recovery of R811H was significantly slower, with $\tau = 2.65 \pm 0.16$ ms for WT ($n=10$) and 5.44 ± 0.44 ms for R811H ($n=7$; $P=0.0359$), which may further exacerbate the phenotype.

The electrophysiological phenotype of $\text{Na}_v1.5$ -R620H was addressed using a similar approach as for $\text{Na}_v1.5$ -R811H (Figure 6). R620H peak currents were similar to WT (Figure 6A and 6B), and none of the kinetic parameters addressed deviated from that of WT (data not shown).

To rule out that the $\text{Na}_v1.5$ -R620H variant could modify the biophysical properties observed for the R811H mutation, we mimicked the compound heterozygosity seen in the patient and coexpressed the mutant proteins in a 1:1 molar ratio (Figure 7). Coexpression of $\text{Na}_v1.5$ -R811H and R620H resulted in a 52% reduction in peak current density at -25 mV compared with WT, whereas R811H alone resulted in a 57% reduction compared with WT, suggesting that the R620H variant does not affect the biophysical properties of R811H.

Electrophysiological Characterization of $\text{Na}_v1.5$ -S1218I

$\text{Na}_v1.5$ -WT or $\text{Na}_v1.5$ -S1218I was expressed in Chinese hamster ovary-K1 cells (Figure 8). WT channels produced robust currents; in contrast, S1218I showed a complete loss of

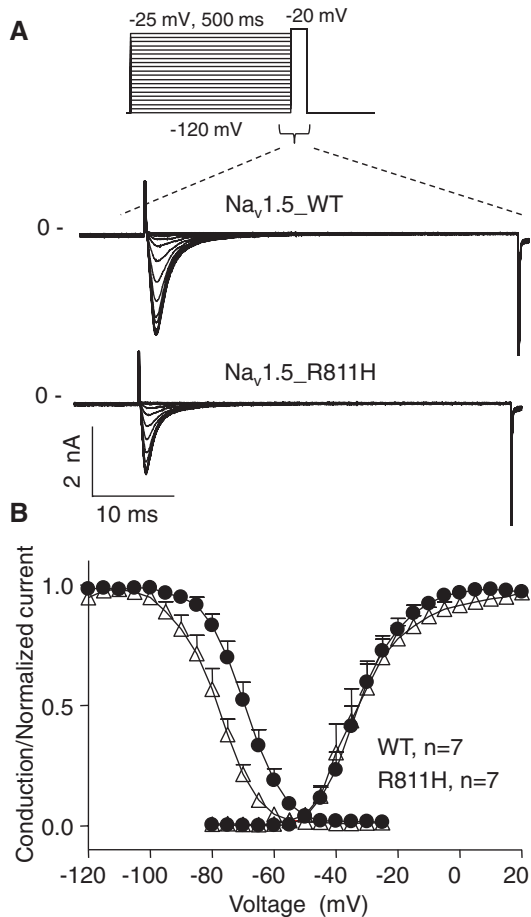


Figure 4. Activation and steady-state inactivation relationship for $Na_v1.5$ wild type (WT; $n=7$) and R811H ($n=7$). Activation curves were calculated by normalizing the currents shown in Figure 3 to $(V_m - E_{K^+})$. **A**, Steady-state inactivation was determined using the protocol shown in the inset. Representative recordings are shown. **B**, Peak currents at the -20 mV step were normalized and plotted against the conditioning potential, and Boltzmann equations were fit to the data points.

function. For WT and S1218I expressed in a 1:1 ratio to mimic the heterozygote state of the patients, peak current density was reduced by $\approx 50\%$, suggesting that the S1218I mutation does

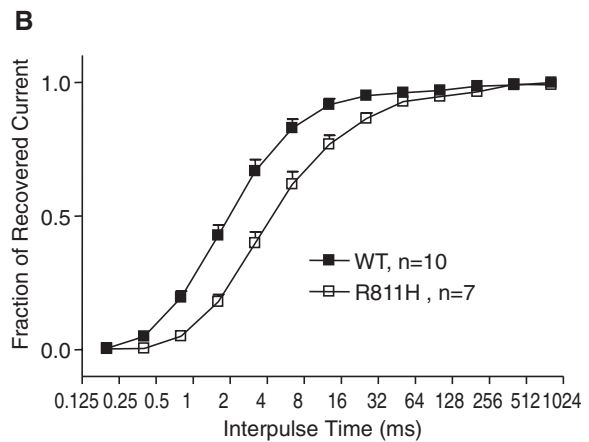
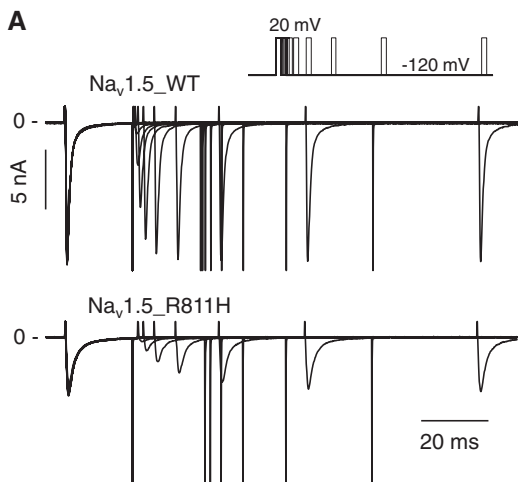


Figure 5. Time-dependent recovery from inactivation for wild type (WT; $n=10$) and R811H ($n=7$). Currents were activated by a 2-pulse protocol from a holding of -120 mV. **A**, Representative recordings. **B**, The fraction of recovered current was plotted as a function of interpulse interval, and single exponential functions were fitted to the data.

not affect WT channels and the phenotype of the patients is because of haploinsufficiency.

Steady-state activation and inactivation were addressed as described for $Na_v1.5$ -R811H, and as expected, no significant difference was observed when comparing WT/S1218I with WT. Similarly, no difference was found in time-dependent recovery from inactivation for WT/S1218I to WT (data not shown).

Discussion

Mutations in *SCN5A* have been implicated in several inherited arrhythmogenic cardiac syndromes, including long-QT syndrome (type 3), BrS (type 1), progressive cardiac conduction disease, atrial fibrillation, and dilated cardiomyopathy.¹⁸ The functional characterization of *SCN5A* mutations and polymorphisms has important implications for the understanding of genotype–phenotype correlations—concordance and discordance—seen in BrS families. Here, we report novel mutations in *SCN5A* in 2 probands with BrS, which cosegregated with the BrS phenotype, and that we characterized in a heterologous mammalian expression system.

Proband 1 had an $Na_v1.5$ compound heterozygous (trans mode) for R811H and R620H mutations. In silico analysis (PolyPhen-2)¹⁹ predicted the *SCN5A* R620H to be benign, while *SCN5A* R811H to be possibly damaging. Family history supported that R811H was likely to be pathogenic, while R620H was likely benign. The proband’s brother who only carried R811H mutation is asymptomatic and has no Brugada pattern on his ECG, whereas the proband’s father who only carried R811H mutation is asymptomatic but has a Brugada pattern on his ECG. Although the R620H was likely benign when inherited without the R811H, having both the R811H and R620H mutations may have produced the abnormal phenotype with BrS. This would not explain the other sudden deaths in the paternal family because the R620H was inherited from the proband’s mother.

$Na_v1.5$ R811H is a novel mutation that occurs in the DII–S4 transmembrane domain of the $Na_v1.5$ channel protein, is not detected in ≈ 400 normal control chromosomes of white

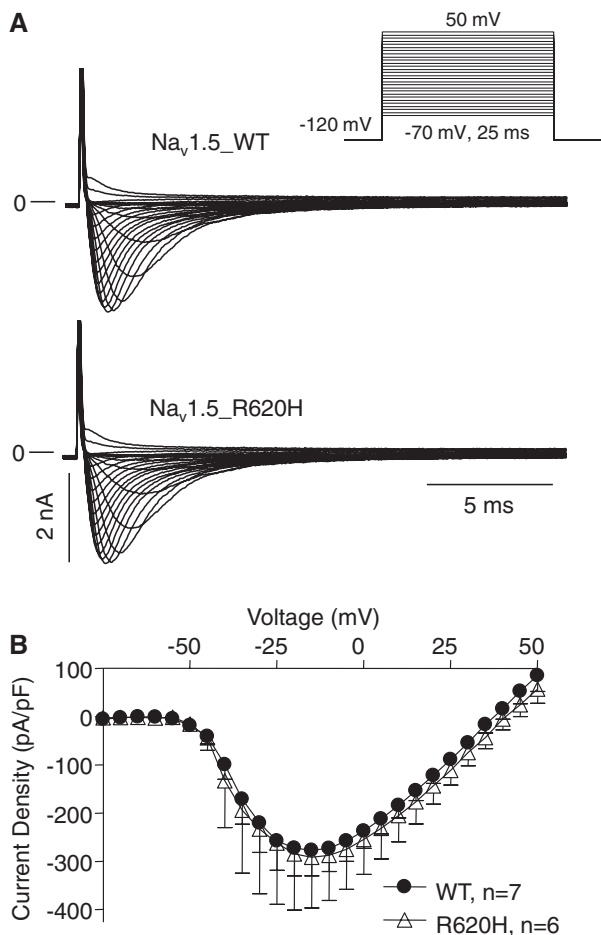


Figure 6. Electrophysiological characterization of R620H. **A**, Representative whole-cell current recordings for Na_v1.5 wild type (WT; n=7) and R620H (n=6) transiently expressed in Chinese hamster ovary-K1 cells. Currents were activated by the depicted voltage-clamp protocol. **B**, Peak current density plotted as a function of voltage.

and black ancestry, and involves a highly conserved amino acid R811 throughout evolution. Mutations affecting surrounding codons (R808P and L812P) have been reported to be associated with BrS.¹⁷ The functional studies of Na_v1.5 R811H in a heterologous expression system suggested that

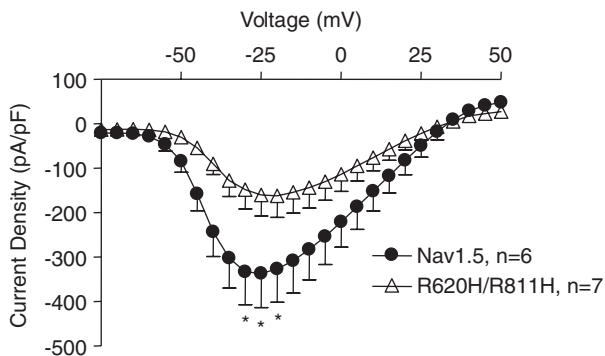


Figure 7. Mimicking the compound heterozygous condition of proband 1. Peak current densities for Na_v1.5 wild type (WT; n=6) and R620H+R811H (n=7) transiently expressed in Chinese hamster ovary-K1 cells. Currents were activated as in Figures 3 and 6.

this mutation decreases the peak Na⁺ current density conferred by Na_v1.5, increases fast and slow Na⁺ current decay time constants, displays a decreased depolarizing shift in voltage dependence of inactivation in the steady-state inactivation recordings, and slows recovery from inactivation compared with WT.

In contrast, Na_v1.5 R620H is a novel mutation in the cytoplasmic linker between DI and DII domains, with no gating kinetic defect and no change in the peak Na current density compared with WT. A recent study suggested that the domain I–II linker might be involved in stabilizing the fast inactivation of Na_v1.5 channels.²⁰ A mutation affecting the same amino acid, R620C, has been reported in association with long-QT syndrome, yet no electrophysiological characterization was performed in that study.¹⁷ Mutations affecting neighboring codons (G615E and L619F) have been reported in patients with BrS.¹⁷ However, in our experiments, R620H did not affect Na_v1.5 function. In line with in silico prediction and family history, coexpressing the 2 variants R620H and R811H mimicking the heterozygous state of the proband rendered a synergistic interaction between them unlikely.

Intriguingly, the proband’s condition was exacerbated with fever. A link between fever and Na_v1.5 function has been suggested earlier.⁷ The authors observed pronounced acceleration of Na_v1.5 activation and fast inactivation kinetics with increased temperature. They also performed cardiac action potential modeling experiments, indicating a shortening of cardiac action potential on fever-induced effect on I_{Na} function.

Proband 2 had a novel Na_v1.5 S1218I mutation. S1218I is a novel mutation in the DIII/S1, resulting in a nonconservative substitution of a small, polar, hydrophilic serine highly conserved through evolution with a larger, nonpolar, hydrophobic isoleucine. In silico analysis (Polyphen-2)¹⁹ predicted that S1218I will be damaging. In line with this, the homozygous Na_v1.5 S1218I had complete loss of function and 50% rescue of the phenotype in the heterozygous state without affecting the biophysical kinetic parameters. The fact that 1 point mutation in the homozygous state abolished the sodium current and the fact that the heterozygous state kinetics were similar to WT but with 50% reduction in peak sodium current density, favor a trafficking defect rather than activation or inactivation gating kinetic defects. Several mutations affecting the DIII of NaV1.5 have been described in BrS (S1219N and E1225K).¹⁷

We have identified and characterized the function of 2 novel *SCN5A* mutations that resulted in partial (R811H) and complete loss of function of I_{Na} (S1218I). The variable phenotypic range of *SCN5A* mutations shows the importance of sodium channel regulation in maintaining normal rhythm. Furthermore, different mutations in *SCN5A* have different impact on I_{Na} aiding the risk assessment of unaffected at-risk family members. The pedigree of proband had 2 *SCN5A* mutations, but our work showed that the R811H led to the Brugada phenotype, whereas the R620H was not pathogenic. Although heterologous expression system for voltage-clamp studies is an approximation of Na_v1.5 function in human cardiomyocytes, this system has been instrumental over the years in understanding how *SCN5A* variants affect arrhythmogenicity.

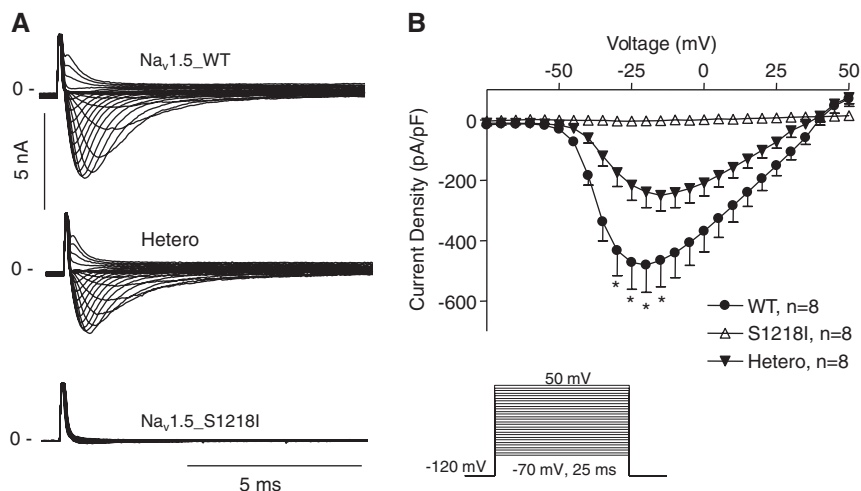


Figure 8. Electrophysiological characterization of S1218L. **A**, Representative whole-cell current recordings for $\text{Na}_v1.5$ wild type (WT), WT expressed with S1218L (1:1 ratio), and S1218L transiently expressed in Chinese hamster ovary-K1 cells. Currents were activated by the depicted voltage-clamp protocol. **B**, Peak current density plotted as a function of voltage, demonstrating a complete loss of function for S1218L.

Conclusions

We describe 2 novel *SCN5A* mutations that result in partial and complete loss of function of I_{Na} . These findings have an important clinical significance and implications by elucidating how different mutations in *SCN5A* have different impact on I_{Na} and help in the risk assessment of unaffected at-risk family members.

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Disclosures

None.

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CLINICAL PERSPECTIVE

In our study, we identified novel mutations in *SCN5A* in patients diagnosed with Brugada syndrome. Of note, 1 proband carried 2 different mutations. Although it is established that mutations in the cardiac sodium channel are associated with Brugada syndrome, there is increasing evidence for the impact of the individual genetic background of a patient. Specifically, other rare or frequent variants may play an important role in the clinical expression of a disease. Our in-depth electrophysiological characterization of each variant shows that these novel mutations result in partial and complete loss of function of I_{Na} , respectively. These findings have clinical implications, in that different mutations in *SCN5A* have different effects on I_{Na} . Dissecting the individual functional phenotype may aid the risk assessment of unaffected at-risk family members.