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Mutations in *SCN10A* Responsible for a Large Fraction of Brugada Syndrome Cases

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Abstract

Objectives—The purpose of this study was to test the hypothesis that *SCN10A* variants contribute to the development of Brugada syndrome (BrS).

Background—BrS is an inherited sudden cardiac death syndrome. Fewer than 35% of BrS probands have genetically identified pathogenic variants. Recent evidence has implicated *SCN10A*, a neuronal sodium channel gene encoding $Na_v1.8$ in the electrical function of the heart.

Methods—Clinical analysis and direct sequencing of BrS-susceptibility genes were performed on 150 probands, family members and >200 healthy controls. Expression and co-immunoprecipitation studies were performed to functionally characterize the putative pathogenic mutations.

Results—We identified 17 *SCN10A* mutations in 25 probands (20 M/5 F); 23 of the 25 (92.0%) displayed overlapping phenotypes. *SCN10A* mutations were found in 16.7% of BrS probands, approaching our yield for *SCN5A* mutations (20.1%). BrS patients with *SCN10A* mutations were more symptomatic and displayed significantly longer PR and QRS intervals than *SCN10A* negative BrS probands. The majority of mutations localized to the transmembrane-spanning regions. Heterologous co-expression of wild-type (WT) *SCN10A* with WT-*SCN5A* in HEK cells caused a near doubling of sodium channel current (I_{Na}) compared with WT-*SCN5A* alone. In contrast, co-expression of *SCN10A* mutants (R14L and R1268Q) with WT-*SCN5A* caused a 79.4% and 84.4% reduction in I_{Na}, respectively. Co-immunoprecipitation studies performed provide evidence for co-association of $Na_v1.8$ and $Na_v1.5$ in the plasma membrane.

Conclusions—Our study identifies *SCN10A* as a major susceptibility gene for BrS, thus greatly enhancing our ability to genotype and risk stratify probands and family members.

Keywords

Electrophysiology; Cardiac Arrhythmias; Brugada syndrome; Cardiac Conduction disease; Sudden Cardiac Death; Genetics

Introduction

The Brugada syndrome (BrS), introduced as a new clinical entity in 1992 (1), is an inherited sudden cardiac death (SCD) syndrome characterized by the appearance of prominent J waves or ST-segment elevation in leads V_1-$V_3$of the electrocardiogram (ECG). An outward shift in the balance of ion channel currents flowing during the early phases of the cardiac action potential have been shown to create the substrate for the development of life-threatening arrhythmias in BrS (2). The syndrome has been associated with 13 genotypes (BrS1 to BrS13) displaying autosomal dominant inheritance (3,4). To date, more than 300 BrS-related mutations in *SCN5A* have been described (5), accounting for the vast majority (>75%) of BrS genotypepositive cases, but only 11-28% of total BrS probands. Approximately 65% of BrS probands remain genetically undetermined. Thus, there is a pressing need to identify new BrS susceptibility genes for the purpose of early diagnosis,

risk stratification, and targeted treatments (6,7). A similar situation is encountered in other inherited cardiac arrhythmia syndromes, including early repolarization syndrome (ERS), cardiac conduction disease (CCD), bradycardia, idiopathic ventricular fibrillation (VF), atrial fibrillation (AF), and right bundle branch block (RBBB).

$Na_v1.8$ (encoded by *SCN10A*), like $Na_v1.5$ (encoded by *SCN5A*), is a tetrodotoxinresistant voltage-gated sodium channel located adjacent to *SCN5A* on human chromosome 3p21–22 (8,9). Until recently, $Na_v1.8$ was principally considered a neuronal sodium channel involved in nociception. The amino acid sequences of human $Na_v1.8$ and $Na_v1.5$ are similar (70.4%). Recent evidence has implicated *SCN10A* in the electrical function of the heart (10-12). Several genome-wide association studies (GWAS) have reported that single nucleotide polymorphisms in *SCN10A* are associated with CCD and arrhythmogenesis (13-21). The present study examines the hypothesis that variations in *SCN10A* contribute to BrS by modulating the expression of $Na_v1.5$ current, the principal cardiac sodium channel. Preliminary results have been reported in abstract form (22).

Methods

Detailed methods are provided in the online supplement.

Clinical analysis and participants

The clinical diagnosis of BrS and ERS was based on criteria provided in the 2005 Consensus Conference document (23) in the case of BrS and criteria suggested in our recent review of the J-wave syndromes in the case of ERS (24).Informed consent was obtained from all patients upon referral to the Masonic Medical Research Laboratory for genetic testing, and patients were tracked anonymously. This study was approved by the regional institutional ethics review board and conducted according to Declaration of Helsinki principles. For each patient, we collected age at time of diagnosis, gender, clinical presentation, family history, and therapy.

Genetic screening and analysis

Genomic DNA was extracted from peripheral blood leukocytes and amplified. All known BrS genes and *SCN10A* were amplified and analyzed by direct sequencing, as previously described (25). The primer sequences for *SCN10A* are shown in Table S1 (Reference Sequence: NM_006514). More than 200 ethnically matched, healthy controls, plus all available online databases for allele frequency, conservation score, and *in silico* pathogenic prediction tools, were probed for prediction of pathogenicity of the variants found.

Co-expression of $Na_V1.5$ and $Na_V1.8$ for co-immunoprecipitation (Co-IP) analysis and electrophysiological investigations

Site-directed mutagenesis was performed on full-length human wild-type (WT) and mutant *SCN10A*-3XFLAG cDNA cloned in pCMV2 vector, the WT *SCN3B* cloned in pCMV6-XL6 vector, and the WT *SCN5A* cloned in pcDNA3.1. Co-immunoprecipitation studies were performed using HEK293 cells transfected with *SCN5A*, *SCN10A* and *SCN3B* plasmids were also used for studies. Total protein was isolated 24 hours after transfection with Lysis buffer

supplemented with protease inhibitors for Co-IP experiment. Membrane currents were measured using whole-cell patch-clamp techniques using TSA201 cells, as previously described (25).

Statistical analysis

Data are presented as mean±SD, unless otherwise noted. For statistical analysis, two-tailed Student's t-test and ANOVA coupled with Student-Newman-Keuls test, were used to compare two groups and more than three groups of continuous variables separately. Chi-square test was used for compare of categorical variables (SigmaStat, Systat Scientific Inc., San Jose, CA). Differences were considered statistically significant at a value of $P<0.05$.

Results

Study population

We systematically evaluated 150 unrelated BrS patients and 17 family members using genetic screening (Table 1). Most patients were male (n=101, 67.3%) with a mean age at diagnosis of 44.5±16.1 years. One hundred and sixteen patients (77.3%) were symptomatic, including 39 (26.0%) who suffered from syncope and 20 (13.3%) who experienced cardiac events, documented as aborted cardiac arrest or SCD. Twenty-nine (19.3%) had a family history of cardiac events or SCD. A Type 1 Brugada ECG pattern, characterized by a prominent J-wave appearing as a coved type ST-segment elevation, was observed spontaneously in 57 patients (38.0%, Figure 1A), appeared after sodium channel blockers in 76 patients (50.7%, Figure 1B) or during fever in the remaining 17 patients (11.3%, Fig. 1C). Some BrS patients also displayed ERS (Fig. 1D), CCD (Fig. 1E), RBBB (Fig. 1F), ventricular tachycardia/ventricular fibrillation (VT/VF), or AF.

Mutation yield and analysis

Overall, 17 putative pathogenic *SCN10A* rare variants [16 missense and 1 frameshift mutation] were identified in 25 probands (Fig. 2A, Tables 2 and 3). Seven family members were positive for *SCN10A* variants. Eleven mutations were identified only once (64.7%), while 6 variants were found in multiple unrelated patients (Fig. 2B). The most frequent mutation was R14L (Fig. 1A), which was carried by 4 BrS probands. The other mutations/rare polymorphisms present in the population were V1697I (3 patients), G1662S (3 patients), I206M (2 patients), I1225T (2 patients), and R1869C (2 patients). Most variants localized to the transmembrane-spanning regions (P-loop 42.3%, S1-S4 23.1% for BrS probands, Fig. 2C).

Among the 25 *SCN10A* mutation or rare variant carriers, 6 carried a secondary mutation in 1 of the 12 known BrS-susceptibility genes (24.0%, Table 2). F938YFSX12, G1406D and N1715T are novel variants in *SCN10A*, not previously reported (Table 3). A majority of missense mutations (13/16) were in highly conserved residues and showed minor allele frequencies (MAF) of 0 to 0.002 in control databases. None were found in more than 400 reference alleles in our healthy controls. All but 1 (T137M) of these 13 mutations were predicted to be damaging by *in silico* prediction tools (Table 3). The MAF of S1337T was 0.0047, that of V1697I was 0.0044, and that of I206M was 0.0046 in our controls. All 4

cases carrying these 3 rare polymorphisms were middle-aged males (31-58 y/o) and 3 were symptomatic.

Overlapping phenotypes of probands with *SCN10A* variants

With a positive proband yield of 16.7%, the prevalence of *SCN10A* in BrS probands is approaching our historical yield for *SCN5A* mutations, which is 20.1% (Fig. 2D). In 25 *SCN10A*$^+$ BrS cases, 23 (92.0%) displayed overlapping phenotypes (Table 2). In cases of BrS with overlapping phenotypes (such as CCD and early repolarization/ER patterns in leads other than V_1-V_3), *SCN10A*$^+$ positive proband yield was greater (Table 1). BrS patients with *SCN10A* mutations were more symptomatic (syncope, SCD, chest pain) and displayed longer PR and QRS intervals (193.4±31.8 ms and 105.7±18.9 ms) than *SCN10A*$^-$ BrS probands (171.5±38.4 ms and 97.3±17.3 ms, $p<0.05$ respectively). No difference in HR, QT, or Bazett corrected QT interval (QTc) was observed. The yield of *SCN10A*$^+$ BrS probands was greater in male (19.8%) than in female (10.2%, Fig. 2F) subjects in general. This difference was not observed in the subgroup of BrS with CCD but was more obvious in BrS cases without CCD. Figure 2E shows yield as a function of age. The yield of probands with spontaneous Type 1 Brugada ECG pattern was 15.8%, which was similar to that in BrS cases unmasked with a sodium channel blocker (14.5%). Interestingly, BrS probands diagnosed during fever showed a much higher yield (5 out of 17; 29.4%) for *SCN10A* variants; all were male.

The average PR interval (PRI) for BrS probands with CCD was 218.3±34.59 ms (maxium PRI, 328 ms). The yield of *SCN10A*$^+$ in this cohort was significantly higher (33.3%) than those without CCD (11.4%; $P<0.01$). Compared with *SCN10A*$^-$ subjects, *SCN10A*$^+$ CCD and BrS cases had a higher incidence of VT/VF, SCD, and chest pain (Table 1).

Also, 24 BrS cases displayed an ER pattern in leads other than V_1-V_3. Seven of these probands and 2 family members were positive for *SCN10A* mutations, including 5 probands with global J-point/wave elevation (ERS3, 71.4%), indicating a higher correlation of *SCN10A* with BrS and ERS compared with BrS phenotype alone. In the case pictured in Fig. 1D, the proband presented with global J-point elevation (ERS/BrS), bradycardia, and a family history of SCD. He and his affected family members carried the same *SCN10A*-G1662S mutation. (Details in the Supplemental Materials).

Among 33 BrS patients presenting with VT/VF, *SCN10A* mutations were identified in 12. BrS appeared spontaneously in 5 cases (41.7%), 2 were unmasked during fever (16.7%) and the rest were unmasked using sodium channel blockers (41.7%). Including those with pediatric bradycardia, the average heart rate of 24 probands with bradycardia and BrS was 51.4±1.7 bpm. *SCN10A* mutations were identified in five cases. Four family members in 3 families also were positive for *SCN10A* mutations (G1662S for 2, R14L for 1, and F938Y FSX12 for 1), indicating clear genetic penetrance. *SCN10A*-S1337T and R1869C were found in 2 AF probands with BrS phenotypes. The *SCN10A*-N1715 mutant carrier presented with BrS and RBBB ECG pattern, an overlapping phenotype recently highlighted by Aizawa et al. (26). (Fig. 1F).

Functional expression studies

For functional characterization, *SCN5A*/WT, *SCN10A*/WT, or *SCN5A*/WT+*SCN10A*/WT were co-expressed with *SCN3B*/WT in HEK293 cells (Fig. 3A). Peak I_{Na}amplitude at -35mV was -462.8±83.2 pA/pF for *SCN5A*/WT+ *SCN3B*/WT. Addition of *SCN10A*/WT yielded a near doubling of peak $I_{Na}$to -859.7±98.9 pA/pF (P<0.01). In contrast, co-expression of *SCN10A*/WT+*SCN3B*/WT alone generated very low amplitude current (-12.2±3.3 pA/pF, P<0.01 compared with the other 2 groups, Fig. 3B). Co-expression of the *SCN10A* mutants, R14L and R1268Q, with *SCN5A*/WT and *SCN3B*/WT caused a major loss of function of I_{Na} (Fig. 3C-I). *SCN10A*-R14L reduced peak I_{Na} density to -177.5±49.5 pA/pF (P<0.01 *vs. SCN10A*-WT) and caused a significant positive shift of half-activation voltage ($V_{1/2}$, P<0.05). *SCN10A*-R1268Q reduced current density to -133.9±36.6 pA/pF (P<0.01 *vs. SCN10A*/WT) with no change in activation parameters. The half-inactivation voltage ($V_{1/2}$) of *SCN10A*-R1268Q was 7.7 mV more negative than that of *SCN10A*-WT when co-expressed with *SCN5A*-WT+*SCN3B*-WT (P<0.05). Recovery from inactivation was similar in the two mutant groups, but both were slower than WT channels (P<0.05 respectively in both $\tau_f$and τ_s). The gating defects caused by *SCN10A*-R14L and *SCN10A*-R1268Q served to reduce sodium channel availability. (Details in Table S2 of the Online Supplement).

Co-IP Study

We examined the capability of $Na_v1.5$ to physically interact with $Na_v1.8$ using Co-IP. The channels were expressed in HEK293 cells either alone or in combination and isolated by pull-down using an antibody to the FLAG on *SCN10A*. Figure 4A shows the protein input for each condition, demonstrating the presence of the transfected proteins under the appropriate conditions. Figure 4B demonstrates the association between $Na_v1.5$ and $Na_v1.8$ when co-expressed (Lane 5, bottom). This interaction was lost when the pull-down antibody was omitted (Lane 4, bottom) and did not occur due to *in vitro* mixing of the protein lysates (Lane 6, bottom).

Discussion

SCN10A in the heart and its role in arrhythmogenesis

SCN5A and *SCN10A* located in close proximity to each other in chromosome 3p22. In 1997, *SCN10A* protein (also referred to as PN3, SNS, and hereafter, $Na_v1.8$) was initially shown to be specifically expressed in rat and human dorsal root ganglia (27). Real-time polymerase chain reaction and immunostaining methodologies have detected a low level of expression of the *SCN10A* gene product in mouse and human heart tissues with somewhat higher levels in the Purkinje system (12,15,18). $Na_v1.8$ immunoreactivity was detected in intra-cardiac neurons and ganglia in human myocardium (28). With *in situ* hybridization method, *SCN10A* displayed a similar distribution pattern Scn5a in mouse hearts (10). These findings notwithstanding, some researchers deny the existence of $Na_v1.8$ in cardiac myocytes. For example, Veldkamp and colleagues reported that *SCN10A* expression modulates cardiac electrical activity primarily by regulating the firing patterns of intracardiac neurons (11). Conflicting data also resulted from other *in vivo* and *in vitro* experimental studies in the animal models (12,15).

The localization, expression level, and function role of $Na_v1.8$ in the heart remain highly controversial. Nonetheless, our results support the conclusion that *SCN10A* variants play a key role in developing arrhythmogenic J-wave syndromes, including both BrS and ERS, likely through a direct effect on Nav1.5-mediated cardiac I_{Na}(Central Illustration). A key role for $Na_v1.8$ in human cardiac electrophysiology is supported by GWAS, showing that *SCN10A* plays an important role in cardiac conduction disease, by influencing PRI and QRS duration, as well as heart rate and arrhythmic risk. Several independent loci within *SCN10A* have been identified, including rs6795970 (13-18), rs6798015 (16,19), rs6800541 (16,20), rs7430477 (16), and rs12632942 (15). A recent genome-wide association study of 312 individuals with BrS and 1,115 controls reported a significant association signal at a *SCN10A* locus rs10428132, providing additional support for a role for *SCN10A* variants, in this case 3[prime]-UTR or intronic, in the development of BrS (21).

Clinical and genetic findings related to *SCN10A*

We identified 17 putative pathogenic *SCN10A* variants in 25 of the 150 BrS probands screened. A positive proband yield of 16.7% is approaching our historical yield of 20.1% for *SCN5A* and a yield of 11% to 28% (21% average) reported in the international compendium of *SCN5A* mutations (5). In our study, as in the international compendium study, there was a male predominance of the BrS phenotype (67% vs.78%). The latter has a similar yield between males and females (20% vs. 22%, respectively).This was not the case in our screen for *SCN10A* mutations, where the yield was greater in the case of males (20% *vs*. 10%).

In our study, 66.7% of *SCN10A* mutations were localized to transmembrane and pore-forming domains; this is in comparison to the nearly 75% reported in the *SCN5A* compendium. Of all BrS-related *SCN10A* variants, one was a frameshift and the rest were missense mutations (94.1%), whereas in the compendium of *SCN5A* mutations, two-thirds were reported to be missense mutations.

In 25 of the cases reported, 6 also were found to carry a second potentially pathogenic BrS mutation (Table 2). As such, the number of *SCN10A* variants that we count as potentially responsible for the clinical phenotype could be an overestimate. This notwithstanding, the 3 mutations in calcium channel genes were found in patients displaying a prolonged PRI (>180 ms) and normal QTc interval, pointing to a clear predominance of the *SCN10A* mutation leading to a loss of function of I_{Na}. The *KCNJ8* mutation likewise was accompanied by a prolonged PRI. The two *SCN5A* mutations were both accompanied by very prolonged PRI (240-280 ms) suggesting that both the *SCN5A* and *SCN10A* variants contributed to the clinical phenotype. Interestingly, the yield of BrS probands unmasked by fever is much higher in the case of *SCN10A* vs. *SCN5A* mutation (29.4% vs. 17.2%, unpublished data from Dan Hu et al.). There was a higher association with SCD and syncope in the case of *SCN10A* vs. *SCN5A* mutations. Also interesting is the larger number of complaints of chest pain in the $SCN10A^+$ group than the $SCN10A^-$ cases, which is not observed when $SCN5A^+$ and $SCN5A^-$ cases are compared.

Greater than 90% of the $SCN10A^+$ BrS subjects presented with mixed phenotypes, the most common of which was CCD. It is not surprising that, as with *SCN5A* mutations (39% when

PR>200 ms vs. 8% when PR<200 ms (6)), the yield of *SCN10A* mutants was much higher in BrS probands with prolonged PRI (31% in PR>200 ms vs.11% in PR<200 ms).

Our observations of a high prevalence of *SCN10A* variants associated with BrS and ERS, most of which: 1) are in amino acid residues that are highly conserved in mammalian species; 2) exhibit a very low MAF in controls; 3) are predicted by *in silico* models to be pathogenic; 4) show good genotype-phenotype correlation in cases in which family pedigrees are available; and 5) show a major loss of function in I_{Na} in the 2 cases in which the variants were functionally co-expressed with *SCN5A*, suggesting that *SCN10A* is an important susceptibility gene for BrS and as well as for other cardiac syndromes including CCD, ERS, AF, VT/VF, RBBB, and bradycardia. *SCN10A* is known to be involved in nociception (29). Our referring physicians did not report altered nociception other than an increased incidence of chest pain.

Mechanisms underlying *SCN10A* modulation of electrical function of the heart

α-subunit interactions have previously been shown to aggravate as well as ameliorate disease phenotypes. The combination of *SCN2A* and *KCNQ2* mutations cause severe seizure manifestations (30), an *Scn8a* mutation has been shown to compensate for haploinsufficiency of *Scn1a* (31), and *SCN9A* mutations are known to modify the severity of *SCN1A*-related Dravet's syndrome (32). A recent BrS study reported a dominant-negative effect of *SCN5A* mutant channels interacting with *SCN5A*-WT channels (33). Given their proximity to one another, *SCN5A* and *SCN10A* may be subject to common regulatory mechanisms, such as transcriptional control by *TBX3 and TBX5* (10).

We hypothesize that *SCN10A* modulates the activity of the canonical cardiac sodium channel encoded by *SCN5A* in the heart. Our co-expression studies provide evidence in support of this hypothesis showing that $Na_v1.5$ and $Na_v1.8$ co-associate when expressed together. The observed functional interaction between $Na_v1.5$ and $Na_v1.8$ may suggest either a direct physical interaction between the two channels or an indirect interaction within a larger protein complex. *SCN10A*-WT causes a gain of function in $Na_v1.5$ current, whereas *SCN10A*-mutants (R14L and R1268Q) cause loss-of-function of $Na_v1.5$ current, which is expected to reduce excitability and lead to development of the arrhythmogenic substrate responsible for BrS and ERS, as well as CCD, VT/VF, AF, RBBB, and bradycardia.

Limitations and future directions

Of the 16 missense mutations uncovered in this study, only 2 were functionally characterized. Despite these limitations, it is important to note that 13 of these variants were totally absent from our own ethnically matched controls and are either absent or negligibly present in all available public databases. Moreover, these mutations are located in highly conserved residues and are predicted to be pathogenic by *in silico* prediction tools.

Our co-immunoprecipitation data pointing to co-association of $Na_v1.5$ and $Na_v1.8$ proteins were performed following co-expression of *SCN5A* and *SCN10A* in HEK cells. Ideally, these studies should be performed in native human ventricular myocytes. This, however, must await the availability of more reliable $Na_v1.8$-specific antibodies. Additional studies

are needed to expand the size of the cohort and to conduct functional expression of WT and mutant *SCN10A* in native myocytes or alternatively in induced pluripotent stem cell-derived cardiomyoctyes.

Conclusions

The findings of this study extend our knowledge of the role of $Na_v1.8$ in the heart and provide an explanation for why *SCN10A* variants cause conduction and rhythm disturbances, some previously identified by GWAS. Our data identify *SCN10A* as a new BrS susceptibility gene and as a potential target for genetic screening and antiarrhythmic intervention. We demonstrate co-localization and co-association of $Na_v1.8$ and $Na_v1.5$ in the plasma membrane and a gain of function of *SCN10A*-WT and loss-of-function of *SCN10A*-mutants on $Na_v1.5$ I_{Na}. BrS males between 11-50 years old, presenting with a prolonged PRI and QRS prolongation, VT/VF, ERS, and/or symptoms (syncope, SCD, chest pain), have the highest probability of carrying an *SCN10A* variant. The spectrum of *SCN10A* arrhythmic phenotypes, including BrS, ERS, CCD, VT/VF, AF, RBBB, and bradycardia, is similar to that of *SCN5A* variants. With a yield of 16.7% for *SCN10A*, a genotype can now be identified by us in more than 50% of BrS probands.

Perspective

Competency in Medical Knowledge

Brugada (BrS) and early repolarization (ERS) syndromes are responsible for ventricular fibrillation (VF) and sudden cardiac death (SCD) of young adults. Fewer than 35% of BrS probands have genetically identified pathogenic variants. The identification of *SCN10A* as a major susceptibility gene for BrS and ERS greatly enhances the capability to risk-stratify probands and family members by genotyping.

Translational Outlook 1

The ability of mutant neuronal sodium channels to cause a loss of cardiac sodium channel activity provides insights into mechanisms by which *SCN10A* variants may contribute to overlap syndromes including BrS, ERS, cardiac conduction disease, and various bradycardia phenotypes

Translational Outlook 2

These findings help to delineate the role of neuronal sodium channels in the electrical function of the heart.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations and Acronyms

BrS	Brugada syndrome
CCD	cardiac conduction defect
Co-IP	co-immunoprecipitation
ERS	early repolarization syndrome
GWAS	genome-wide association studies
INa	sodium channel current
MAF	minor allele frequency

PRI	PR interval
SCD	sudden cardiac death
WT	wild type

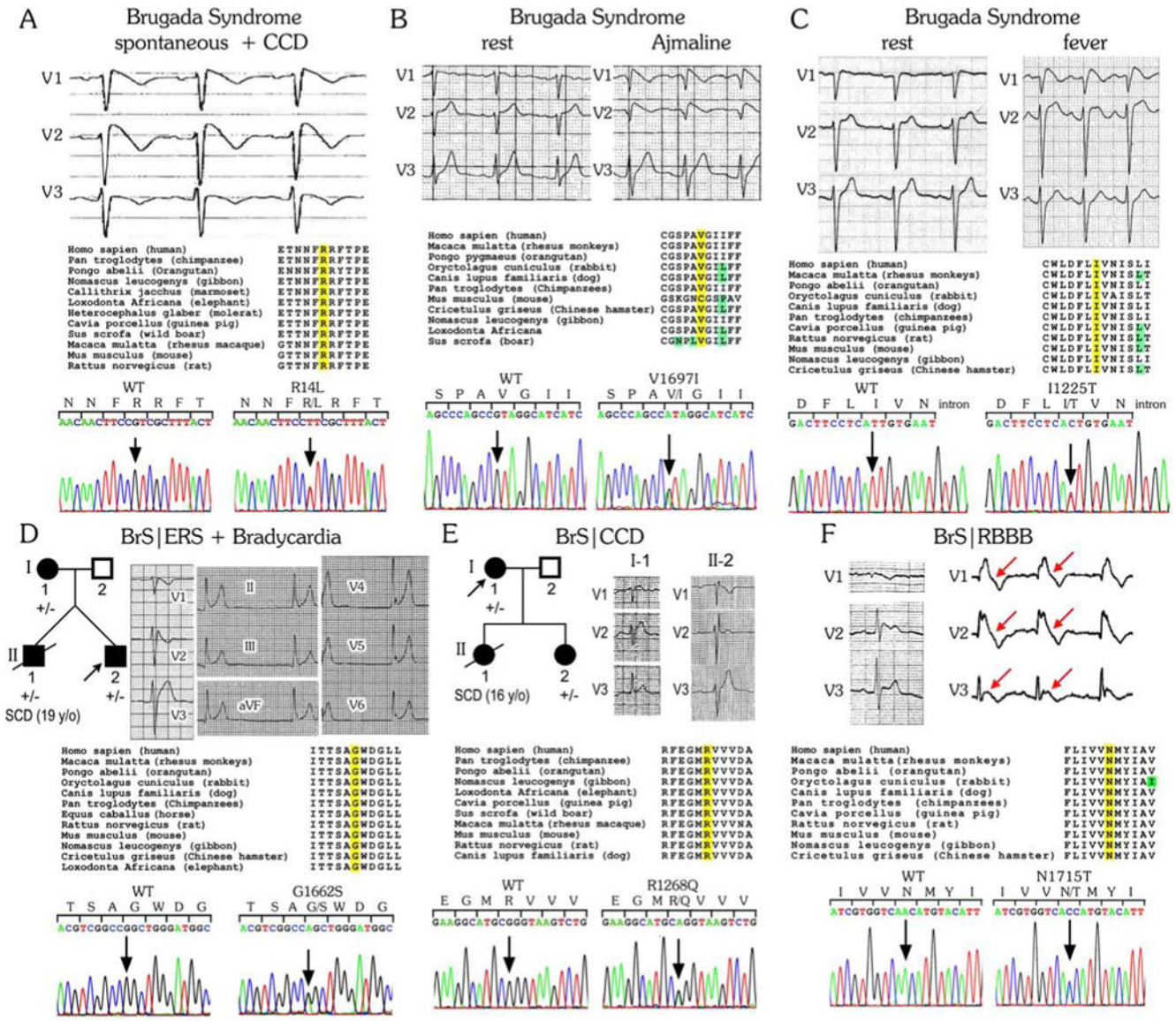


Figure 1. Representative Cases of the Different Brugada Syndrome (BrS) Phenotypes Associated with the *SCN10A* Mutations/Rare Variants Identified

Each panel shows the ECG phenotype, amino acid alignments of the mutated residue position in a number of mammalian species, and DNA chromatogram of wild-type (WT) and mutant *SCN10A*. For the pedigrees in panels **D&E**, +/- denotes heterozygous for the mutation; circles represent female subjects and squares represent male subjects. The arrow denotes the proband. Clinically affected and unaffected subjects are labeled as black and white, respectively. CCD: cardiac conduction disease; ERS= early repolarization syndrome; RBBB= right bundle branch block; SCD= sudden cardiac disease.

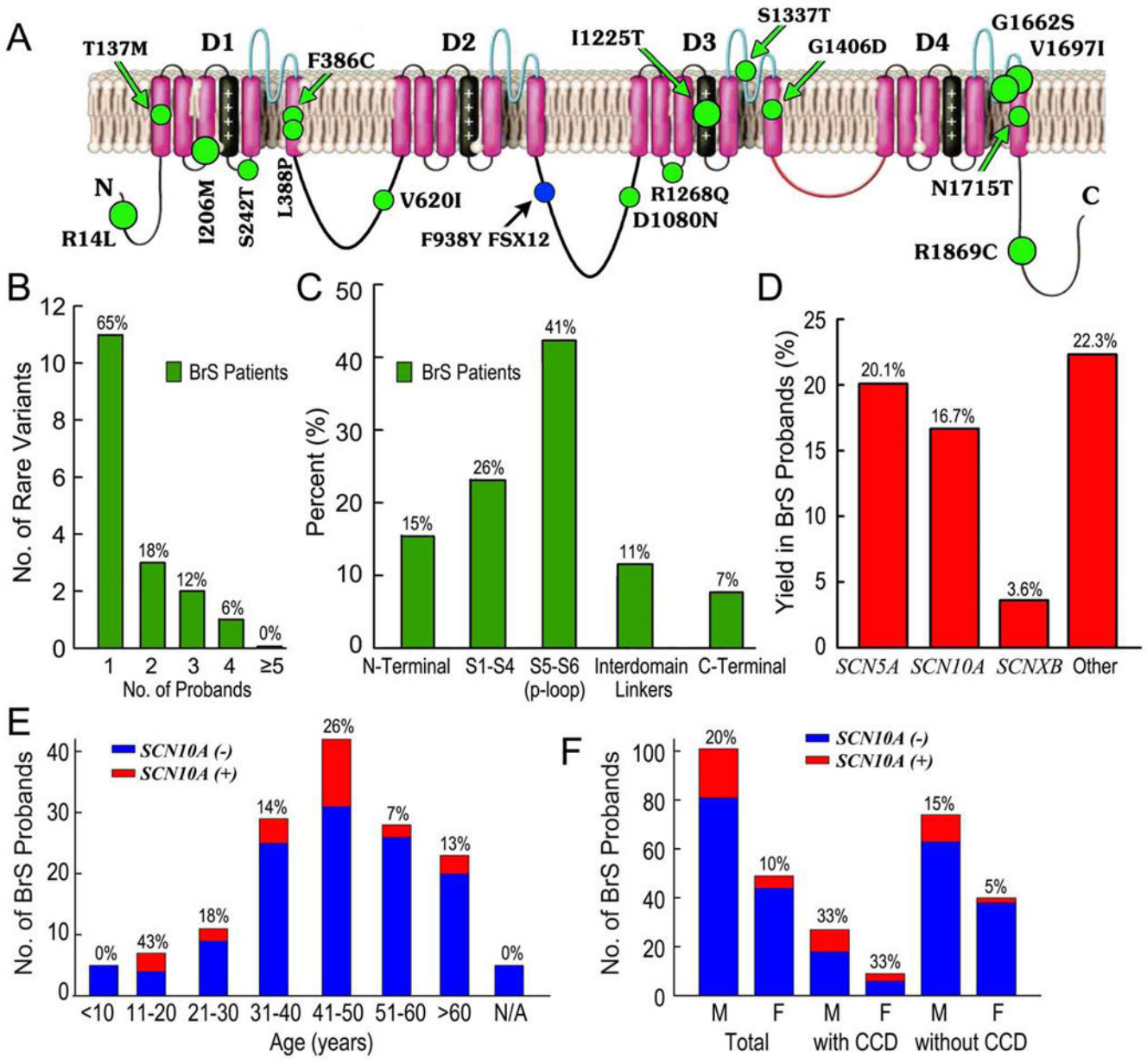


Figure 2. Clinical and Genetic Prevalence of *SCN10A* Mutations/Rare Variants in probands with Brugada Syndrome (BrS) Identified in the Present Study

A: Schematic showing topology of $Na_v1.8$, the pore-forming α subunit encoded by *SCN10A* and location of putative BrS-causing variants. **B**: Frequency distribution of *SCN10A* mutations/rare variants in BrS cases (green). **C**: Percentage of mutations/rare variants in BrS cases (green) by location. **D**: Mutation detection yield by gene in Masonic Medical Research Laboratory BrS cases. **E&F**: Bar graph showing age and gender distribution of BrS cases. CCD= cardiac conduction disease.

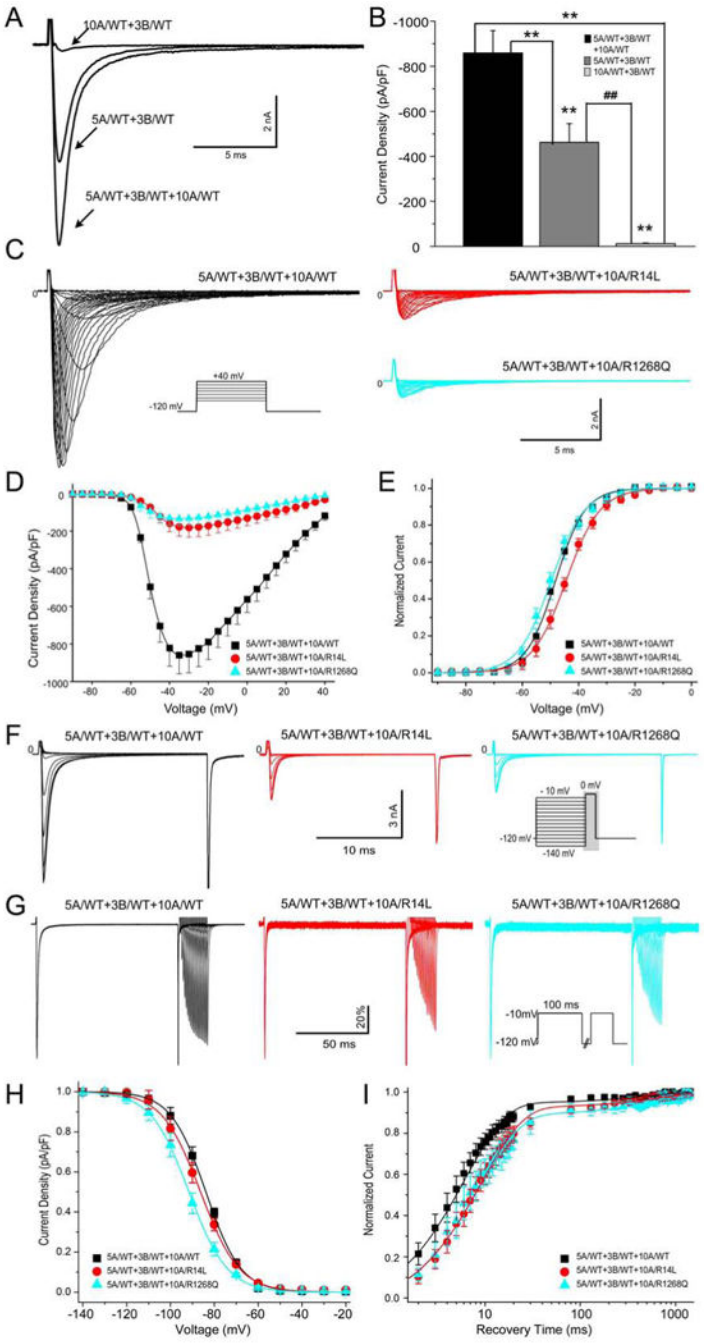
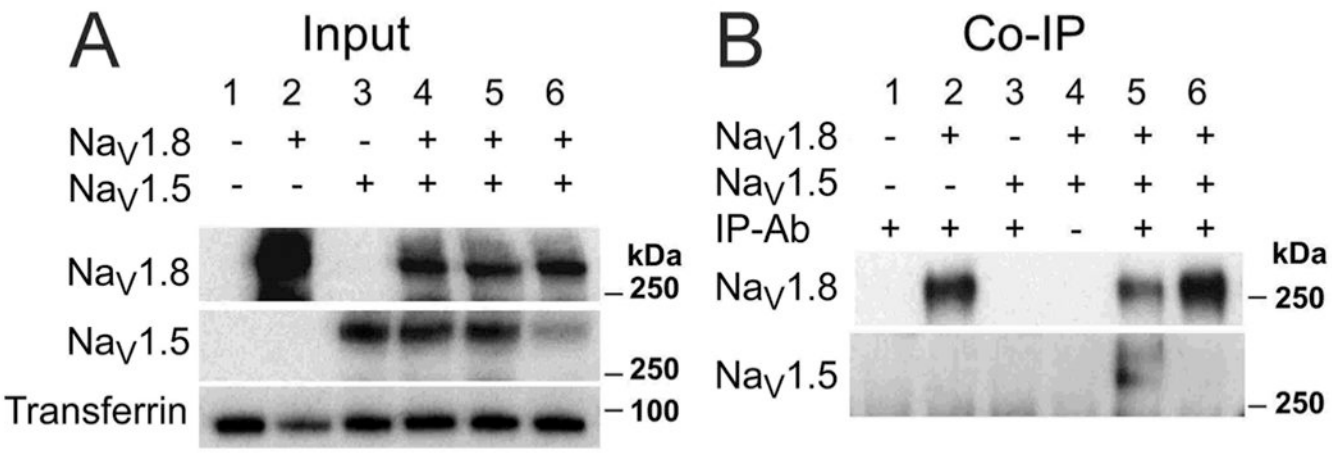


Figure 3. Electrophysiology Effect of *SCN10A* on Cardiac Sodium Channel Current (I_{Na}) when Co-Expressed with *SCN5A* and *SCN3B* in HEK293 Cells

A&B: Superimposed traces and bar graph depicting peak I_{Na} recorded from co-expression of *SCN10A*/wild type (WT)+*SCN3B*/WT, *SCN5A*/WT+*SCN3B*/WT and *SCN5A*/WT +*SCN10A*/WT+*SCN3B*/WT. **P<0.01 vs. *SCN5A*/WT+*SCN10A*/WT+*SCN3B*/ WT, ##P<0.01 vs. *SCN5A*/WT+*SCN3B*/WT. **C-E**: Representative I_{Na} traces, current-voltage relationship and voltage dependence of activation for *SCN10A*/WT, *SCN10A/R14L* and *SCN10A/R1268Q* when co-expressed with *SCN5A*/WT+*SCN3B*/WT. **F&G**: Representative

steady-state inactivation and recovery traces recorded from WT and mutant channels. **H&I**: Boltzmann distributions of voltage-dependent channel inactivation and recovery curve with a double-exponential fit for the 3 groups. All related values and the number of cells used are presented in Table S2 in the Supplemental Materials.



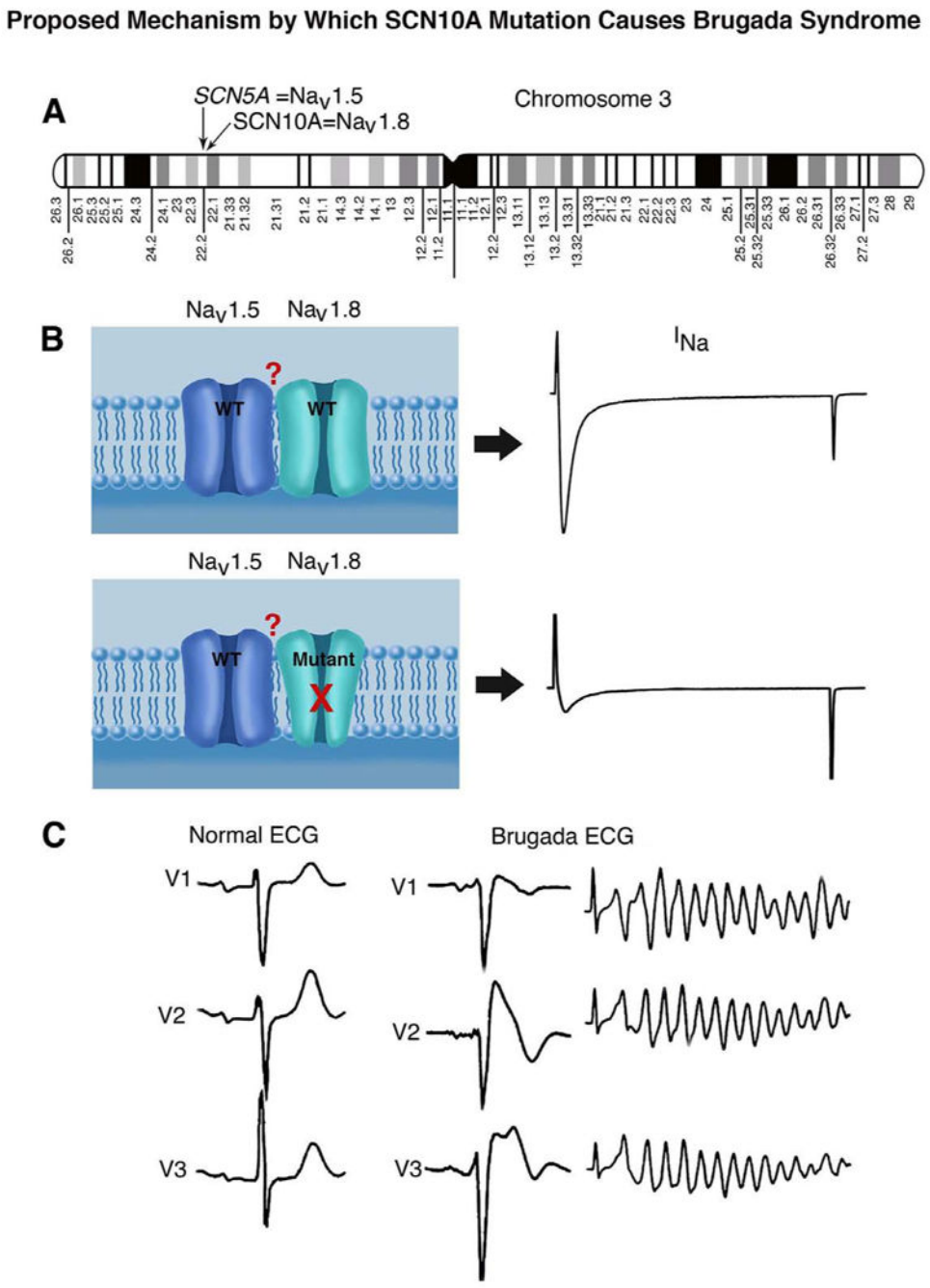


Figure 4. Representative Experiments Demonstrating Physical Interaction between $Na_V1.8$ and $Na_V1.5$

Panel **A** shows protein input and panel **B** shows protein isolated by the antibody pull-down co-immunoprecipitation (Co-IP). Lanes 1-6 correspond to the following experimental conditions: (1) non-transfected, (2) $Na_V1.8$ expressed alone, (3) $Na_V1.5$ expressed alone, (4 and 5) $Na_V1.8$ and $Na_V1.5$ co-expressed, (6) mixed lysates from lanes 3 and 4. Panel **B** shows the pull-down of $Na_V1.5$ is specific to $Na_V1.8$ cellular co-expression.

Central Illustration: SCN5A and SCN10A, genes encoding cardiac and neuronal sodium channels, are found in close proximity on chromosome 3 (A). Our study suggests that mutations in SCN10A can lead to a loss of function in sodium channel current (INa) and thus contribute to the manifestation of Brugada syndrome (BrS), a sudden cardiac death syndrome. The data suggest physical association of the two channel proteins (NaV1.5 and NaV1.8) in the plasma membrane (B). Our study identifies SCN10A as a major susceptibility gene for BrS, thus greatly enhancing our capability to genotype and risk stratify probands and family members (C).

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Table 1

Demographics of Patients with Brugada Syndrome (BrS)

	Probands with BrS			Probands with BrS and CCD		
	Overall	***SCN10A*$^+$**	***SCN10A*$^-$**	**Overall**	***SCN10A*$^+$**	***SCN10A*$^-$**
Patient Demographics						
Number of probands	150	25 (Yield 16.67%)	125	36	12 (Yield 33.33%)	24
Family history	29 (19.33%)	4 (16.00%)	25 (20.00%)	8 (22.22 %)	3 (25.00%)	5 (20.83%)
Age for diagnosis	44.48±16.10	42.50±13.56	44.83±16.61	44.75±19.92	43.25±15.15	45.50±22.18
Male	101 (67.33%)	20 (80.00%)	81 (64.80%)	27 (75.00%)	9 (75.00%)	18 (75.00%)
Female	49 (32.67%)	5 (20.00%)	44 (35.20 %)	9 (25.00%)	3 (25.00%)	6 (25.00 %)
with CCD	36 (24.00%)	12 (48.00%)	24 (19.20%)	36 (100.00%)	12 (100.00%)	24 (100.00%)
ER†	24 (16.00%)	8 (32.00%)	16 (12.80%)	8 (22.22%)	3 (25.00%)	5 (20.83%)
bradycardia	24 (16.00%)	5 (20.00%)	19 (15.20%)	10 (27.78%)	3 (25.00%)	7 (29.17%)
VT/VF	33 (22.00%)	12 (48.00%)	21 (16.80%)	12 (33.33%)	7 (58.33%)	5 (20.83%)
AF	13 (8.67%)	2 (8.00%)	11 (8.80%)	1 (2.78%)	0 (0.00%)	1 (4.17%)
Symptom						
Asymptomatic	34 (22.67%)	1 (4.00%)	33 (26.40%)	9 (25.00%)	1 (8.33%)	8 (33.33%)
Syncope	39 (26.00%)	10 (40.00%)	29 (23.20%)	10 (27.78%)	4 (33.33%)	6 (25.00%)
SCD	20 (13.33%)	6 (24.00%)	14 (11.20%)	3 (16.67%)	3 (25.00%)	3 (12.50%)
chest pain	11 (7.33%)	5 (20.00%)	6 (4.80%)	3 (8.33%)	3 (25.00%)	0 (0.00%)
other symptoms‡	54 (36.00%)	12 (48.00%)	42 (33.60%)	11 (33.33%)	4 (33.33%)	8 (33.33%)
Electrocardiograms						
HR (bpm)	73.82±16.58	72.58±16.35	74.10±16.69	68.07±17.77	72.00±21.39	65.11±15.31
PR (ms)	175.97 ± 38.23	193.44±31.79*	171.47±38.42	218.29±34.59	217.42±24.20	218.77±39.66
QRS (ms)	98.84±17.82	105.72±18.85*	97.26±17.28	103.47±19.34	107.67±24.01	101.38±16.72
QT (ms)	378.94±42.43	384.44±38.67	377.70±43.30	398.28±44.45	388.67±48.18	403.08±42.71
QTc (ms)	414.40±36.22	416.05±37.42	414.04±36.11	416.29±42.24	420.40±49.82	414.23±38.92

*P<0.05 compared between *SCN10A*$^+$ and *SCN10A*$^-$groups.

† Early repolarization (ER) pattern other than V_1-V_3

‡ Other symptoms include palpitation, dizziness, sleep apnea, coma, et al.

AF: atrial fibrillation; CCD: cardiac conduction disease; HR: heart rate; SCD: sudden cardiac death; VT/VF: ventricular tachycardia/ventricular fibrillation.

Table 2

Clinical and Genetic Characteristics of Affected Probands

No.	General Information			Symptom				Arrhythmia						Genetic Rare Variants		ECG					Treatment
	Age for Dx (y/o)	Sex	FH*	Syncope	SCD	Chest pain	Other†	BrS/ERS	ER Pattern other than V1-V3	CCD	Bradycardia	VT/VF	AF	*SCN10A*	Other suspected gene	HR (bpm)	PR (ms)	QRS (ms)	QT (ms)	QTc (ms)	
1	65	M	N	N	N	N	Y	BrS (Spontaneous BrS/RBBB)	N	Y (IAVB)	N	Y (induced)	N	N1715T	N	71	220	72	426	463	ICD
2	50	F	Y	N	N	Y	Y	BrS (Spontaneous)	I, avL	Y (IAVB)	N	N	N	R14L	N	73	234	112	402	443	
3	42	F	N	Y	N	Y	Y	BrS (Spontaneous)	N	N	N	Y (induced)	N	F938YFSX12	N	68	160	120	400	426	ICD
4	44	M	N	Y	N	N	N	BrS (Spontaneous)	N	N	N	Y (induced)	N	G1662S	N	79	190	120	380	436	Quinidine
5	20	M	N	Y	Y	N	N	BrS/ERS3 (Spontaneous/Procainamide+)	II, III, avF	N	N	N	N	S242T	N	67	170	105	376	396	
6	19	M	N	N	N	Y	N	BrS (Procainamide+)	N	N	N	Y (induced)	N	R14L	N	66	185	113	397	416	
7	36	M	N	N	N	N	Y	BrS (Ajmaline+)	N	N	N	Y (spontaneous)	N	G1406D	N	73	150	110	400	442	ICD
8	19	M	Y	Y	Y	Y	Y	BrS/ERS3 (Flecainide+)	II, III, avF, V4-V6	Y (IAVB)	Y	Y (spontaneous)	N	G1662S	N	44	200	120	410	352	
9	39	M	N	Y	N	N	N	BrS(Ajmaline+)	I, avL	N	N	N	N	I206M/V1697I	N	78	168	80	340	388	
10	46	M	N	Y	N	N	Y	BrS (Procainamide+)	N	N	N	N	Y	S1337T	N	69	148	105	418	448	
11	37	M	N	N/A	N/A	N/A	N/A	BrS (Ajmaline+)	N	Y (I AVB)	N	N	N	T137M	N	59	205	72	350	348	
12	49	F	Y	Y	N	N	Y	BrS (Spontaneous)	N	Y (IAVB)	N	Y (spontaneous)	N	R1268Q	N	79	170-200	102	380	436	ICD
13	44	M	N/A	N	N	N	Y	BrS (fever+)	N	N	N	N	N	I1225T	N	80	180	110	350	404	
14	27	M	N	N	Y	N	N	BrS (fever+)	N	Y (IAVB)	N	Y (during fever)	N	G1662S	N	120	220	120	300	387	ICD, Metoprolol
15	44	M	N	Y	N	N	N	BrS (fever+)	N	Y (IAVB)	N	Y (during fever)	N	I1225T	N	103	200	116	400	525	ICD
16	56	M	N/A	N	N	N	Y	BrS (fever+)	N	N	N	N	N	L388P	N	86	180	112	350	418	
17	31	M	Y	N	N	N	N	BrS (Ajmaline+)	N	Y (IAVB)	N	Y (induced)	N	V1697I	N	75	200	120	360	403	
18	65	M	Y	Y	Y	N	N	BrS/ERS3 (Spontaneous)	II, III, avF	N	Y	Y (spontaneous)	N	R14L	N	54	194	94	400	378	ICD, Amiodarone
19	49	M	N/A	N	N	N	Y	BrS (fever+)	AvL	N	N	N	N	R14L	N	88	130	110	340	411	
20	58	M	N	Y	N	N	N	BrS (Ajmaline+)	N	Y (IAVB)	N	N	N	I206M/V1697I	*CAC NB2B*	84	210	100	344	408	ICD, Metoprolol
21	46	F	N	N	Y	N	N	BrS/ERS3 (Spontaneous)	I, II, III, avF, V5-V6	Y (IAVB)	Y	N	N	V620I	*SCN5A*	53	280	84	432	406	ICD
22	65	M	N	N	Y	N	N	BrS (Flecainide+)	N	Y (IAVB)	Y	Y (spontaneous)	N	F386C	*CACNA1C*	55	200	116	484	464	ICD
23	28	M	N	N	N	Y	N	BrS (Spontaneous/Procainamide+)	N	Y (IAVB)	N	N	N	D1080N	*SCN5A*	71	240	158	376	409	ICD
24	43	M	N	N	Y	N	Y	BrS (Procainamide+)	N	N	N	N	Y	R1869C	*KCNJ8*	64	192	84	372	385	
25	47	F	N	N	N	N	Y	BrS/ERS3 (Ajmaline+)	II, III, avF, V4-V6	N	Y	N	N	R1869C	*CACNB2B*	56	18 0	88	42 4	40 8	
Total	42.5 ± 13.6	F5/M20(80%)	5 (20%)	10 (40%)	7 (28%)	5 (20%)	12 (48%)	25 (100%)	8 (32%)	12 (48%)	5 (20%)	12 (48%)	2 (8%)	25		72.6 ± 16.3	193.4 ± 31.8	105.7 ± 18.9	384.4 ± 38.67	416.1 ± 37.4	ICD-11 (44.0%)

No.	General Information			Symptom				Arrhythmia						Genetic Rare Variants		ECG					Treatment
	Age for Dx (y/o)	Sex	FH*	Syncope	SCD	Chest pain	Other†	BrS/ERS	ER Pattern other than V1-V3	CCD	Brady cardia	VT/VF	AF	*SCN10A*	Other suspected gene	HR (bpm)	PR (ms)	QRS (ms)	QT (ms)	QTc (ms)	
SCN 10A†	41.2 ± 13.7	F3/M16(84%)	5 (26%)	9 (47%)	4 (21%)	4 (21%)	10 (53%)	19 (100%)	6 (32%)	8 (42%)	2 (10%)	11 (58%)	1 (5%)	19		75.4 ± 16.8	18 6.0 ± 26.9	10 5.9 ± 15.6	37 7.8 ± 32.9	416.9 ± 40.9	ICD-7 (36.4%)
SCN 10A and other gene†	47.8 ± 12.8	F2/M4(67%)	0 (0 %)	1 (17%)	3 (50%)	1 (17%)	2 (33%)	6 (100%)	2 (33%)	4 (67%)	3 (50%)	1 (17%)	1 (17%)	6		63. 8 ± 12.0	21 7.0 ± 37.0	10 5.0 ± 28.7	40 5.3 ± 51.0	413.3 ± 26.4	ICD-4 (66.7%)

AF: atrial fibrillation; AVB: atrioventricular block; BrS: Brugada syndrome; CCD: cardiac conduction disease; Dx: diagnosis; ER: early repolarization; ERS3: Type 3 early repolarization syndrome; F: female; HR: heart rate; ICD: implantable cardioverter-defibrillator; M: male; RBBB: right bundle branch block; SCD: sudden cardiac death; VT/VF: ventricular tachycardia/ventricular fibrillation.

*FH: Family history of cardiac events/unexplained sudden death.

†Other symptoms, including palpitation, dizziness, coma, *et al*.

Table 3

Summary of SCN10A Rare Variants Associated with Brugada Syndrome.

Rare Variant	Reported ID	Exon	Type	Change in nucleotide	Change in amino acid	Codons	Location	Global MAF (1000genome)	Global MAF (ESP)	Number of Unrelated individuals	Number of Family members	SIFT-Score	SIFT-Prediction	Polyphen-Score	Polyphen-Prediction	Conservation (phast Cons)*	Conservation (GERP)†
R14L	rs141207048	1	missense	p.Arg14 Leu	c.41G>T	CGT/CTT	N-terminal	0.002	0.002153	5	2	0	deleterious	0.998	probably damaging	0.987	2.32
T137M	rs148663098	3	missense	p.Thr137 Met	c.410C>T	ACG/ATG	DI-SI	0	0.000077	1	0	1	tolerated	0.022	benign	0.077	3.26
I206M	rs74717885	5	missense	p.Ile206 Met	c.618A>G	ATA/ATG	DI-SIII	0.048	0.01161	4	0	0.01	deleterious	0.082	benign	0.001	-0.84
S242T	rs140288103	6	missense	p.Ser242 Thr	c.724T>A	TCA/ACA	DI-S4/S5	0	0.000231	1	0	0	deleterious	0.999	probably damaging	0.997	4.69
F386C	rs78555408	9	missense	p.Phe386 Cys	c.1157T>G	TTC/TGC	DI-S6	0.002	N/A	1	1	0	deleterious	1	probably damaging	1	5.21
L388P	rs199734710	9	missense	p.Leu388 Pro	c.1163T>C	CTG/CCG	DI-S6	0.001	0.000231	1	0	0	deleterious	1	probably damaging	1	5.21
V620I	rs151303346	12	missense	p.Val620 Ile	c.1858G>A	GTC/ATC	DI/DII	0	0.000384	1	0	0.03	deleterious	0.999	probably damaging	0.818	4.6
F938YFSX12	N/A	16	Frame shift	p.Phe938 Tyr FsX12	c.2813-2814 delinA	‡	DII/DIII	N/A	N/A	1	1	N/A	N/A	N/A	N/A	N/A	N/A
D1080N	TMPESP3 38765035	18	missense	p.Asp1080 Asn	c.3238G>A	GAC/AAC	DII/DIII	0	0.000077	1	0	0.17	tolerated	0.967	probably damaging	0.75	4.82
I1225T	rs139638446	20	missense	p.Ile1225 Thr	c.3674 T>C	ATT/ACT	DIII-S4	0	0.000461	2	0	0	deleterious	1	probably damaging	0.992	4.45
R1268Q	rs138832868	21	missense/near-splice	p.Arg1268 Gln	c.3803G>A	CGG/CAG	DIII-S4/S5	0.001	0.002076	1	1	0	deleterious	0.741	probably damaging	1	4.14
S1337T	rs11711062	22	missense	p.Ser1337 Thr	c.4009T>A	TCC/ACC	DIII-S5/S6	0.001	0.004536	1	0	0.61	tolerated	0.059	benign	0	-9.34
G1406D	N/A	24	missense	p.Gly1406 Asp	c.4217G>A	GGC/GAC	DIII-S6	N/A	N/A	1	0	0	deleterious	0.999	probably damaging	1	5.11
G1662S	rs151090729	27	missense	p.Gly1662 Ser	c.4984G>A	GGC/AGC	DIV-S5/S6	0.001	0.001307	3	2	0	deleterious	1	probably damaging	0.969	5.38
V1697I	rs77804526	27	missense	p.Val1697 Ile	c.5089G>A	GTA/ATA	DIV-S5/S6	0.004	0.010072	4	0	0.3 7	tolerated	0.098	benign	0.138	0.24
N1715T	N/A	27	missense	p.Asn1715 Thr	c.5144A>C	AAC/ACC	DIV-S6	N/A	N/A	1	0	0	deleterious	1	probably damaging	1	4.19
R1869C	rs141648641	27	missense	p.Arg1869 Cys	c.5605C>T	CGC/TGC	C-terminal	0	0.000923	2	0	0	deleterious	1	probably damaging	0.945	5.09

N/A: not available; 1000 genome: the 1000 Human Genome Project Database; ESP: Exome Sequencing Project; MAF: the Minor-Allele Frequency.

*Conservation (phastCons): a number between 0 and 1 that describes the degree of sequence conservation among 17 vertebrate species; these numbers are downloaded from the University of California Santa Cruz Genome site (http://genome.ucsc.edu/).

†Conservation (GERP): The Genomic Evolutionary Rate Profiling (GERP) score was obtained from the GERP website in September of 2011. It ranges from -12.3 to 6.17, with 6.17 being the most conserved.

‡T2813 & C2814 are replaced by an A, causing a frameshift resulting in a stop codon 12 AA later.