NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

BACKGROUND—Brugada syndrome (BrS) is a common heritable channelopathy. Mutations in the *SCN5A*-encoded sodium channel (BrS1) culminate in the most common genotype.

OBJECTIVE—This study sought to perform a retrospective analysis of BrS databases from 9 centers that have each genotyped >100 unrelated cases of suspected BrS.

METHODS—Mutational analysis of all 27 translated exons in *SCN5A* was performed. Mutation frequency, type, and localization were compared among cases and 1,300 ostensibly healthy volunteers including 649 white subjects and 651 nonwhite subjects (blacks, Asians, Hispanics, and others) that were genotyped previously.

RESULTS—A total of 2,111 unrelated patients (78% male, mean age 39 ± 15 years) were referred for BrS genetic testing. Rare mutations/variants were more common among BrS cases than control subjects (438/2,111, 21% vs. 11/649, 1.7% white subjects and 31/651, 4.8% nonwhite subjects, respectively, $P < 10^{-53}$). The yield of BrS1 genetic testing ranged from 11% to 28% ($P = .0017$). Overall, 293 distinct mutations were identified in *SCN5A:* 193 missense, 32 nonsense, 38 frameshift, 21 splice-site, and 9 in-frame deletions/insertions. The 4 most frequent BrS1-associated mutations were E1784K (14×), F861WfsX90 (11×), D356N (8×), and G1408R (7×). Most mutations localized to the transmembrane-spanning regions.

CONCLUSION—This international consortium of BrS genetic testing centers has added 200 new BrS1-associated mutations to the public domain. Overall, 21% of BrS probands have mutations in *SCN5A* compared to the 2% to 5% background rate of rare variants reported in healthy control subjects. Additional studies drawing on the data presented here may help further distinguish pathogenic mutations from similarly rare but otherwise innocuous ones found in cases.

Keywords

Brugada syndrome; Genetic testing; Ion channels; Sodium channel; Sudden death

Introduction

Brugada syndrome (BrS) is a rare heritable arrhythmia syndrome characterized by an electrocardiographic (ECG) pattern consisting of coved-type ST-segment elevation in the right precordial leads V_1 through V_3 (often referred to as a type-1 Brugada ECG pattern) and an increased risk for sudden cardiac death (SCD).[1,2] The penetrance and expressivity of this autosomal-dominant disorder is highly variable, ranging from a lifelong asymptomatic course to SCD during the first year of life. The syndrome is thought to account for up to 4% of all SCDs and 20% of unexplained sudden death in the setting of a structurally normal heart;[3] however, some patients display a more benign course. BrS is generally considered a disorder involving young male adults, with arrhythmogenic manifestation first occurring at an average age of 40 years, with sudden death typically occurring during sleep.[4] However, BrS has also been demonstrated in children and infants as young as 2 days old and may serve as a pathogenic basis for some cases of sudden infant death syndrome.[3]

Since the disorder's sentinel clinical and ECG description in 1992 by Drs. Pedro and Josep Brugada,[5] *SCN5A*-encoded cardiac sodium channel loss-of-function mutations have been shown to confer the pathogenic basis for an estimated 15% to 30% of BrS, currently representing the most common BrS genotype and classified as Brugada syndrome type 1 (BrS1).[6–8] Loss-of-function mutations in *SCN5A* reduce the overall available sodium current (I_{Na}) through either impaired intracellular trafficking of the ion channel to the plasma membrane, thereby reducing membrane surface channel expression, or through altered gating properties of the channel. Gain-of-function *SCN5A* mutations cause a clinically and mechanistically distinct arrhythmia syndrome, long-QT syndrome type 3 (LQT3). Interestingly, some identical *SCN5A* mutations may provide either a loss-of-function BrS1-

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

phenotype or a gain-of-function LQT3-phenotype, depending on the individual host. In fact, LQT3/BrS/conduction-disorder *SCN5A* overlap syndromes do exist within single large families.[9,10]

After a decade of genetic testing by research laboratories worldwide, BrS genetic testing has made the transition from discovery to translation to clinical implementation with the availability of clinical BrS1 genetic testing (since 2004 in North America and even earlier in Europe), which provides comprehensive open-reading frame and canonical splice site mutational analysis of *SCN5A*. However, it must be recognized that nearly 2% of healthy Caucasians and 5% of healthy nonwhite subjects also host rare missense *SCN5A* variants, leading to a potential conundrum in the interpretation of the genetic test results.[11] Distinguishing pathogenic mutations from rare harmless genetic variants is of critical importance in the interpretation of genetic testing and the management of genotype-positive BrS patients.

Presently, there are over 100 BrS1-associated mutations publicly available (http://www.fsm.it/cardmoc). We sought to assemble an international compendium of putative BrS1-associated mutations through a retrospective analysis of BrS genomic databases from 9 reference centers throughout the world (5 Europe, 3 United States, 1 Japan) that have each genotyped >100 unrelated cases of clinically suspected BrS. Such a compendium may illuminate further key structure–function properties and provide a foundational building block for the development of algorithms to assist in distinguishing pathogenic mutations from similarly rare but otherwise innocuous ones.

Methods

Study population

A retrospective analysis of BrS databases from 9 centers throughout the world that have each genotyped >100 unrelated cases of clinically suspected BrS was performed. In total, 2,111 unrelated patients (78% male, mean age 39 ± 15 years) were referred for *SCN5A* genetic testing (Table 1). For the purpose of this compendium of identified mutations, only minimal demographic information for each center's cohort, such as the average age and range of age at diagnosis and the number of male and female subjects was provided. The specific age and gender were collected for mutation-positive patients. A sample was accepted for genetic testing if the referring physician had made a clinical diagnosis of either possible or definite BrS. An ECG was not always available for each patient. Although DNA samples were accepted for analysis based on a referral diagnosis of BrS, several of the international centers did collect and examine 12-lead ECGs to confirm the presence of an ECG pattern consistent with BrS.

Mutational analysis

Patient genomic DNA was analyzed for mutations in all 27 translated exons, including splice sites and adjacent regions, of the *SCN5A*-encoded cardiac sodium channel $Na_V1.5$ using a combination of polymerase chain reaction (PCR), either denaturing high-performance liquid chromatography or single-stranded conformation polymorphism and DNA sequencing.[12] In addition, frequency, location, and mutation type of *SCN5A* genetic variation found among 1,300 ostensibly healthy volunteers,[11,13] including 649 white subjects and 651 nonwhite subjects (black, Asian, and Hispanic), was analyzed and compared with the possible BrS1-associated mutations. To be reported in this compendium as a possible BrS1-associated mutation, the case mutation must have been absent among all 1,300 control subjects who underwent comprehensive mutation scanning. Further, each reference center examined a local set of control samples from usually 200 to 400 additional unrelated, healthy individuals to

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

determine the presence or absence of each possible case mutation observed in patients from their respective geographical region.

Mutation nomenclature

All possible BrS1-associated mutations were denoted using the accepted Human Genome Variation Society's guidelines for nomenclature.[14] The nucleotide and amino acid designations were based on the *SCN5A* transcript NM_198056.2. For example, the missense mutation E1784K would indicate the wild-type amino acid (E = glutamic acid) at position 1784 is replaced by lysine (K). Frameshift mutations resulting from nucleotide insertions or deletions were annotated using the F861WfsX90 format, which indicates that the wild-type phenylalanine (F) at position 861 is altered to a tryptophan (W) followed by 89 miscoded amino acids prior to a termination codon (X) 90 residues from the beginning of the altered reading frame.

A substitution of either the first or the last 2 nucleotides of a particular exon has the capacity to alter proper mRNA splicing, regardless of whether the nucleotide substitution codes for a different amino acid (missense mutation) produces a stop codon (nonsense mutation) or does not alter the open reading frame at all (i.e., a synonymous or silent single-nucleotide substitution).[15–17] As such, mutations involving this exonic portion of the splice site were considered as possible splicing mutations in this study and annotated as either missense/splicesite, nonsense/splice-site, or silent/splice-site mutations to distinguish them from intronic mutations predicted to disrupt splicing.

Topological placement of the mutations was assigned using a combination of Swissprot (http://ca.expasy.org/uniprot/) and recent studies of the linear topologies for the sodium channel pore-forming alpha subunit.[18–20] The Swissprot database provides generally accepted residue ranges corresponding with each ion-channel region and specialized subregions. For Nav1.5, mutations were localized to either the N-terminus (amino acids 1 to 126), interdomain linker (IDL I-II, aa 416-711, IDL II-III, aa 940-1200, and IDL III-IV, aa 1471-1523), transmembrane/linker (Domain I, aa 127-415, Domain II, aa 712-939, Domain III, aa 1201-1470, Domain IV, aa 1524-1772), or C-terminus (aa 1773-2016). The transmembrane-spanning region was further subdivided into S1 through S4 (DI S1-S4/S5, aa 127-252, DII S1-S4/S5, aa 712-841, DIII S1-S4/S5, aa 1201-1336, and DIV S1-S4/S5, aa 1524-1659) and S5 through S6, the pore region and selectivity filter of the channel (DI S5-S6, aa 253-415, DII S5-S6, aa 842-939, DIII S5-S6, aa 1337-1470, DIV S5-S6, aa 1660-1772).

Defining terminology: variant versus mutation

For the purposes of this compendium, a variant will be defined as any change to the wild-type sequence, whether it is in case or control subjects. Mutations will be identified as rare, caseonly (absent in the 1,300+ healthy volunteers) variants that are possibly pathogenic. Variants identified with a minor allele frequency (MAF) >0.5% among the 1,300 healthy control subjects will be termed common polymorphisms. If the MAF is <0.5%, these variants will be termed uncommon/rare polymorphisms.

Defining a variant as a possible BrS1-causative mutation

To be considered as a possible BrS1-causing mutation, the variant must disrupt either the open reading frame (i.e., missense, nonsense, insertion/deletion, or frameshift mutations) or the splice site (polypyrimidine tract, splice acceptor, or splice donor recognition sequences). In addition to the exonic splice sites described above, the acceptor splice site was defined as the 3 intronic nucleotides preceding an exon (designated as IVS−1, −2, or −3) and the donor splice site as the first 5 intronic nucleotides after an exon (designated as IVS+1, +2, +3, +4, or +5).[17] Additionally, single-nucleotide substitutions (namely, a purine [A or G] for a pyrimidine [C

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

or T]) within the polypyrimidine tract immediately preceding the acceptor splice-site may be causative.[17] As such, some pyrimidine-to-purine substitutions in this region of the intron have been included as potentially pathogenic. For example, an IVS-5 cytosine (C) that falls within the polypyrimidine tract and is substituted by an adenine (A) that would predictably disrupt the polypyrimidine tract and consequently result in aberrant splicing would be included as a possible pathogenic mutation. Hence, single-nucleotide substitutions that obviously did not change the open reading frame (i.e., synonymous single-nucleotide polymorphisms) or those outside of the splice site recognition sequence were not included in either case or control subjects for this study.

Additionally, to be considered as a possible BrS1-causing mutation, the nonsynonymous variant must have been absent in all published databases listing the *SCN5A* channel common polymorphisms and previously published reports or compendia of rare control variants, e.g., those found in over 2,600 reference alleles (Table 2) derived from over 1,300 ostensibly healthy adult volunteers.[11,13] As such, the sole or concomitant presence of a common polymorphism such as H558R-SCN5A or a rarer polymorphism such as A572D-SCN5A would not by definition warrant the annotation of possible BrS1-associated mutation and would not be counted toward the assignment of compound or multiple mutation status to an individual in this compendium. This does not imply that common and rare polymorphisms may not possibly modulate the BrS1 phenotype.

Results

Overall, 2,111 unrelated patients (78% male, average age at testing 39 ± 15 years) were referred for BrS genetic testing across 9 testing centers (Table 1). As expected, rare *SCN5A* missense mutations were far more common among BrS cases (438/2,111, 21%) than similarly rare genetic variants were among control subjects (43/1,300 [11/649, 1.7% white subjects and 31/651, 4.8% nonwhite subjects], $P < 10^{-55}$). The yield differed significantly across centers ($P = .0017$; $chi^2 = 24.7$, degrees of freedom (df) = 8), ranging from 11% (centers 8 [14/130] and 9 [12/111]) to 28% (center 7 [43/153], $P = .0017$ for the 9 centers) (Table 1, Figure 1). There was no significant difference in yield between male (324/1,520, 21.3%) and female (112/439, 25.5%, $P = .07$) subjects. Of the 438 *SCN5A* mutation-positive cases, 13 (3%) harbored multiple mutations (Table 3). All 13 were male and trended toward younger age at diagnosis (29.7 ± 16.2 years) than male subjects with a single mutation (39.2 ± 14.4 years, $P = .07$).

Overall, 293 distinct, possible BrS1-associated mutations (200, 68% novel to this cohort), absent in 2,600 reference alleles, were identified in the 438 genotype-positive cases, 225 (77%) of which were identified only once (Table 4, Figure 2). Only 68 mutations were found in multiple unrelated patients. The 4 most frequent BrS1-associated mutations were E1784K (14 patients), F861WfsX90 (11 patients), D356N (8 patients), and G1408R (7 patients) (Table 4, Figure 2). Two-thirds of the unique mutations were missense mutations (193), whereas the remaining third (100) involved radical mutations (38 frameshift, 32 nonsense, 21 splice-site, and 9 in-frame insertions or deletions) (Table 4, Figure 3).

Of the 293 unique mutations, 208 (71%) localized to one of the 4 transmembrane-spanning regions (DI, DII, DIII, or DIV), 54 (18%) localized to an IDL (31 in IDL I-II, 17 in IDL II-III, and 6 in IDL III-IV), 17 (6%) localized to the C-terminus, and 14 (5%) localized to the N-terminus (Table 4, Figure 4). The majority of patients with a single *SCN5A* mutation (313/425, 74%) hosted a mutation that localized to the transmembrane region of the channel, with 31% (133/425) having a mutation that localized to either DI S1-S4, DII S1-S4, DIII S1-S4, or DIV S1-S4 and 42% (180/425) having a mutation that localized to the S5, P-loop, and S6 regions containing the pore and selectivity filter of the sodium channel (DI S5-S6, DII S5-S6, DIII S5-

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

S6, or DIV S5-S6) (Table 4, Figure 4). In fact, compared with the topological location for the rare control variants, there was a strong predilection for a patient's possible BrS1-associated mutation to localize to the channel's transmembrane (S1-S4 6.3% vs. 0.2%, $P < 10^{-24}$) and pore-forming segments (S5-S6 8.5% vs. 0.5%, $P < 10^{-30}$) (Figure 5). Twenty-eight patients (1.3%) had their possible BrS1-associated missense mutation localizing to the DI-DII or DII-DIII linker domains, where the vast majority of the rare missense SCN5A variants, which were discovered among the ostensibly healthy control subjects, reside. The yield for the linker regions was 3% for cases compared with 1.7% for healthy subjects (P = NS) (Figure 5).

Interestingly, 10 (3.4%) of the mutations, identified in 29 patients of this BrS compendium, have been identified previously in cases of long-QT syndrome (LQTS): G615E, L619F, E1225K, P1332L, L1501V, R1623Q, I1660V, V1667I, T1779M, including the most common BrS1-associated mutation, E1784K, observed in this BrS compendium. Five of the 10 have been functionally characterized previously, with L619F,[21] P1332L,[22] R1623Q,[23] and E1784K[24] producing channel abnormalities consistent with an LQT3 phenotype and G615E[25] producing a wild-type-like SCN5A channel.[10]

Discussion

Since the first report by Chen et al[26] in 1998, a little over 100 unique *SCN5A* mutations have been implicated as possibly causative for BrS1. Previous small cohort studies have indicated that the prevalence of *SCN5A* mutations in BrS is roughly 15% to 20%, and possibly as high as 40% in cases of familial BrS. In 2000, Priori et al[6] reported a 15% yield with respect to *SCN5A* mutations among 52 unrelated patients. In 2002, these investigators extended their analysis to 130 probands (20% with a family history of sudden unexplained death) and identified an *SCN5A* mutation in 22%.[7] Schulze-Bahr et al[8] reported a 14% yield among 44 unrelated BrS patients, who are included in the current compendium.

Here, through this international multicenter study, we provide an expanded compendium of 293 unique (200 novel) BrS1-associated *SCN5A* mutations derived from over 2,100 unrelated patients referred for BrS genetic testing. The overall yield was 21% and ranged from 11% to 28% among the 9 centers. The differences in yield between the centers may be due to technical differences in mutational analysis methods used among laboratories, but it more likely reflects phenotypic differences among cohorts. For example, the cohorts with the lowest yield may have a preponderance of sporadic cases compared with familial cases. In 2003, Schulze-Bahr et al[8] reported that although none of their 27 sporadic BrS cases hosted an *SCN5A* mutation, 38% of their index cases with clearly familial BrS were positive. The number of sporadic versus familial BrS patients for each center's cohort is currently unknown. Alternatively, some of the lower yield centers may have accepted a greater proportion of weaker clinical cases for BrS genetic testing because no particular litmus test was demanded before acceptance of a sample.

Eight of the 9 centers represent research-based genetic testing laboratories, where most often the cohorts for such laboratories are composed of phenotypically robust cases of BrS. However, 1 center (center 5 in Table 1) represents a clinical laboratory offering the commercially available, fee-based genetic test for both BrS1 and LQTS. In this setting, the level of clinical suspicion and the usage of the genetic test by the referring physician are unknown for each patient sample submitted. Among their first 195 BrS referral cases submitted for clinical genetic testing, 22.6% were identified with a possible pathogenic *SCN5A* mutation, which is in line with the higher point estimate of a 20% prevalence of *SCN5A* mutations among clinically strong cases of BrS patients,[7] and consistent with the noncommercial centers in this compendium. In contrast, this laboratory has reported a yield of 36% for the first 2,500 consecutive unrelated LQTS referral cases tested compared with a yield of 75% among clinically strong cases of

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

LQTS.[27] These observations suggest that in clinical practice, prescribing cardiologists are submitting higher-probability BrS cases for BrS1 genetic testing compared with LQTS.

In this compendium, 3% of the genotype-positive patients hosted multiple putative pathogenic *SCN5A* mutations, absent in control subjects. Akin to genotype–phenotype observations in LQTS,[28] patients hosting multiple *SCN5A* mutations were younger at diagnosis (29.7 ± 16 years) than those having a single mutation. Nearly half of the 13 cases (all male) with multiple mutations were younger than 25 years of age, with the youngest presenting at 2 years of age. Whether carriers of multiple mutations had a more severely expressed phenotype, such as having more multiple syncopal events, aborted cardiac arrest, or stronger family history of SCD, than single-mutation carriers is unknown.

Mutations in this compendium overwhelmingly represent "private" mutations, meaning they were seen only once. Nearly 80% of the 293 unique mutations were identified in a single unrelated case. Fewer than 20 mutations were seen in more than 3 unrelated BrS patients. However, nearly 10% of the 438 unrelated *SCN5A* mutation-positive patients hosted 1 of 4 mutations: E1784K (14 patients), F861WfsX90 (11 patients), D356N (8 patients), and G1408R (7 patients). In this compendium, more than one-third of the genotype-positive patients had radical or non-missense mutations (i.e., frameshift, nonsense, and splicing errors) that represent extremely high-probability case mutations (only 1 such variant was found in 1 of the 1,300 healthy volunteers) and would predictably cause a significant loss of sodium channel function through a mechanism of haploinsufficiency. Recently, Meregalli et al[29] reported that BrS patients with truncation mutations, caused by radical mutations, had a more severe phenotype characterized as a higher propensity for syncope and prevalence of SCD among young first-degree relatives, than those BrS patients hosting missense mutations functionally characterized with ≤90% peak sodium current reduction.

The *SCN5A*-encoded cardiac voltage-gated sodium channel (Nav1.5), which is responsible for the initial fast upstroke of the cardiac action potential and accordingly plays a vital role in the excitability of myocardial cells and the proper conduction of the electrical pulsation of the heart, consists of 4 homologous domains (DI-DIV) that are connected by intracellular linkers. Each domain contains 6 transmembrane-spanning segments (S1-S6). Although case mutations were scattered throughout Nav1.5, there was clustering of putative BrS1-associated mutations whereby nearly three-fourths localized to the transmembrane and pore-forming domains compared with <20% of the rare variants found among the control subjects. Further, nearly 10% of patients with clinically suspected BrS had a mutation localizing to the channel's pore/selectivity filter (segments S5 and S6 and the interconnecting P-loops) compared with <0.5% of the control subjects. Because of the extreme rarity of pore-localizing missense variants among the healthy control subjects, such missense mutations found in cases are high probability BrS1-causative mutations. However, whether BrS1 patients with pore mutations behaved more poorly than those with mutations localizing to other domains was unable to be gleaned in this study.

In contrast, only 25 patients hosted a single missense mutation residing in either the DI-DII or DII-DIII linker domains. The observed preponderance (over 50% of which localize to these 2 IDLs) of the 42 unique rare missense variants identified in the healthy control subjects suggest that some of the 21 IDL localizing, possible BrS1-associated missense mutations that were identified in 25 cases may in fact represent false positives.[11,13] Despite being absent in over 1,300 control subjects, either cosegregation or functional studies on these DI-DII and DII-DIII linker-localizing mutations should be considered before upgrading their status from a rare variant of uncertain significance to a probable BrS1-causative missense mutation. For example, 1 of the 21 missense mutations, G615E, has been previously characterized as having no

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

significant changes in current density or kinetics compared with wild type, casting some doubt on its level of causality.[25]

Whereas loss-of-function mutations in SCN5A have been shown to serve as a pathogenic basis for BrS, gain-of-function mutations in SCN5A provide the pathogenic substrate for LQT3, a clinically and mechanistically distinct arrhythmia syndrome from BrS. Interestingly, 10 mutations identified in this BrS compendium have been implicated previously in LQTS, including the most commonly observed mutation, E1784K. In fact, E1784K represented the most commonly observed SCN5A mutation (4/26, ~15%) among a cohort of 541 unrelated LQTS patients.[28] Although the clinical phenotype for the patients hosting 1 of these 10 mutations could have been assigned incorrectly, it is far more likely that these represent overlap syndrome/mixed phenotype syndrome–associated *SCN5A* mutations.

For example, E1784K represents the quintessential example of a cardiac sodium channel mutation with the capacity to provide for a mixed clinical phenotype of LQT3, BrS, and conduction disorders.[30] Makita et al[30] reported recently a high prevalence of LQT3/BrS/conduction phenotype overlap among 41 E1784K carriers from 15 kindreds of diverse ethnic background. Of the 41 cases, 93% displayed a prolonged QTc, 22% with a diagnostic indicator (ST-segment elevation or positive provocation test) of BrS, and 39% had sinus node dysfunction. Functional characterization of mutant E1784K sodium channels displayed unique biophysical and pharmacological properties consistent with other mutations that yield a mixed phenotype, including a negative shift of steady-state sodium channel activation and enhanced tonic block in response to sodium channel blockers, leading to an additional BrS/sinus node dysfunction phenotype in conjunction with a prolonged QTc.[30] This particular functional effect may influence the pharmacological management of patients with E1784K-SCN5A disease.[30]

Similarly, Bezzina et al[31] in 1999, described an LQT3/BrS overlap phenotype in a large 1795insD-SCN5A mutation–positive 8-generation kindred characterized with a high incidence of sudden nocturnal death, QT-interval prolongation, and Brugada ECG. Whether or not the 9 other mutations, identified here in BrS patients and elsewhere in patients purported to have LQTS, have a similar functional characteristic that provides a substrate for producing a mixed/overlapping clinical phenotype remains to be seen.

Given that *SCN5A* remains the most common BrS genotype despite accounting for only 20% of BrS, genetic heterogeneity of the disease is evident and the role of genetic background in the pathophysiology of BrS is important.[32] Recently, mutations in the glycerol-3-phosphate dehydrogenase 1–like protein encoded by *GPD1L* have been shown to affect trafficking of the sodium channel to the plasma membrane, thus reducing overall sodium current, giving rise to the BrS phenotype.[33] Recently, mutations involving the L-type calcium channel alpha and beta subunits encoded by the *CACNA1C* and *CACNB2b* genes, respectively, were implicated in nearly 11% of BrS cases.[34] Other minor causes of BrS include mutations in the sodium channel beta 1 subunit encoded by *SCN1B* and in a putative beta subunit of the transient outward potassium channel (I_{to}) encoded by *KCNE3*.[35,36] The most recent gene associated with BrS is the *SCN3B*-encoded beta-3 subunit of the cardiac sodium channel.[37]

A number of these minor BrS-susceptibility genes function in part as sodium channel interacting proteins (ChIPs). The cardiac sodium channel is now understood to be a part of a macromolecular complex, with numerous ChIPs regulating its expression, localization, and function. As exemplified by *GPD1L, SCN1B*, and *SCN3B*, other genes, which encode other sodium ChIPs whose disruption would portend a loss of function sodium channel phenotype, would warrant examination as candidate BrS disease or disease-modifying genes. These 6 minor BrS-susceptibility genes (*GPD1L, CACNA1C, CACNB2B, SCN1B, SCN3B*, and *KCNE3*) have not been examined among the remaining 1,673 *SCN5A*-negative unrelated cases

represented in this compendium, but it is predicted that these minor genes will explain <10% of this cohort. Thus, the majority of BrS still remains genetically elusive.

Study limitations

For the purpose of this compendium of identified mutations, only minimal demographic information from each center's cohort was made available because the focus was on the prevalence, spectrum, and localization of *SCN5A* mutations among suspected cases of BrS rather than an attempt to establish any particular genotype–phenotype correlates. Nevertheless, there is significant clinical value due to the data in aggregate. For example, the rare missense mutations seen numerous times among these cases referred for BrS genetic testing, and still not among the control subjects, indicate high-probability BrS-associated mutations.

Furthermore, despite the lack of clinical information, the physical distribution of mutations/variants among cases and control subjects is very telling, whereby the power in numbers can come from the series of mutations/variants clustering in a region, even if the individual mutations/variants have only been observed rarely. For example, even without cosegregation data or in vitro function analyses, the next rare, nonmissense mutation as well as the next transmembrane-localizing missense mutation represent high-probability pathogenic substrates. Conversely, those rare single amino acid substitutions localizing to the domain I-II and II-III linkers should be buttressed with either clinical cosegregation data or in vitro functional data before being upgraded from this list of possible deleterious mutations to highly probable deleterious mutations.

Additionally, this analysis focused on only identifying coding and splicing region single-nucleotide mutations and small insertion/deletion mutations by molecular techniques that often do not detect larger rearrangements, insertions, and deletions. *SCN5A* promoter region mutations, deep intronic mutations, epigenetic methylation mutations, and large genomic rearrangements, all of which could predictably produce a loss-of-function phenotype, would have escaped detection by the mutational analyses performed herein. However, such alterations are quite uncommon when compared with changes in the known coding sequences of the genes.

Despite these limitations, this compendium of nearly 300 distinct BrS-associated mutations provides key observations that may assist in the further interrogation of the cardiac sodium channel biology and serve as a foundation for the development of algorithms to assist in distinguishing pathogenic mutations from similarly rare but otherwise innocuous ones.

Conclusion

Since the sentinel discovery of BrS as a cardiac channelopathy in 1998, our genomic understanding of this potentially lethal disorder has matured from a phase of discovery to one of translational medicine. This international consortium of BrS genetic testing centers has tripled the catalog of possible BrS1-associated mutations with the addition of 200 new mutations to the public domain and has provided a template to draw upon for further genetic testing interpretation and biological inquiry.

Acknowledgments

Support for data analysis for this project was provided by the Mayo Clinic Windland Smith Rice Comprehensive Sudden Cardiac Death Program (Dr. Ackerman), grant HL47678 from the National Institutes of Health (Dr. Antzelevitch), New York State and Florida Free and Accepted Masons, the GIS Institut des Maladies Rares, the AFM (ANR-06-MRAR-022, PG, Dr. Schott), The Health Sciences Research Grants (H18, Research on Human Genome, 002) and the Research Grant for the Cardiovascular Diseases (21C-8) from the Ministry of Health, Labor, and Welfare of Japan (Dr. Shimizu), The Fondation Leducq Trans-Atlantic Network of Excellence Grant (05 CVD 01, Preventing Sudden Death, Dr. Schott), ANR grant ANR/-05-MRAR-028-01 (Dr. Schott), grant from the Fondation pour la

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

recherche Medicale (Dr. Schott), FIS-ISCiii (Dr. Brugada), CNIC (Dr. Brugada), Ramon Brugada Sr. Foundation (Dr. Brugada), Leducq Foundation, grant 05 CVD, Alliance against Sudden Cardiac death (Drs. Wilde, Schott, and Schulze-Bahr), and Deutsche For-schungsgemeinschaft (Dr. Schulze-Bahr). All mutational analyses performed in this study were conducted at individual centers. Dr. Ackerman is a consultant for PGxHealth. Intellectual property derived from Dr. Ackerman's research program resulted in license agreements in 2004 between Mayo Clinic Health Solutions (formerly Mayo Medical Ventures) and PGxHealth (formerly Genaissance Pharmaceuticals).

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

ABBREVIATIONS

BrS	Brugada syndrome
BrS1	Brugada syndrome type 1
ChIPs	sodium channel interacting proteins
ECG	electrocardiographic
IDL	interdomain linker
I_{Na}	available sodium current
LQT3	long-QT syndrome type 3
LQTS	long-QT syndrome
MAF	minor allele frequency
PCR	polymerase chain reaction
SCD	sudden cardiac death

References

1. Chen PS, Priori SG. The Brugada syndrome. J Am Coll Cardiol 2008;51:1176–1180. [PubMed: 18355655]
2. Meregalli PG, Wilde AAM, Tan HL. Pathophysiological mechanisms of Brugada syndrome: depolarization disorder, repolarization disorder, or more? Cardiovasc Res 2005;67:367–378. [PubMed: 15913579]
3. Antzelevitch C, Brugada P, Borggrefe M, et al. Brugada syndrome: report of the second consensus conference. Heart Rhythm 2005;2:429–440. Erratum: Heart Rhythm 2005;2:905. [PubMed: 15898165]
4. Tester DJ, Ackerman MJ. Cardiomyopathic and channelopathic causes of sudden unexplained death in infants and children. Annu Rev Med 2009;60:69–84. [PubMed: 18928334]
5. Brugada P, Brugada J. Right bundle branch block, persistent ST segment elevation and sudden cardiac death: a distinct clinical and electrocardiographic syndrome. A multicenter report. J Am Coll Cardiol 1992;20:1391–1396. [PubMed: 1309182]
6. Priori SG, Napolitano C, Gasparini M, et al. Clinical and genetic heterogeneity of right bundle branch block and ST-segment elevation syndrome: a prospective evaluation of 52 families. Circulation 2000;102:2509–2515. [PubMed: 11076825]
7. Priori SG, Napolitano C, Gasparini M, et al. Natural history of Brugada syndrome: insights for risk stratification and management. Circulation 2002;105:1342–1347. [PubMed: 11901046]
8. Schulze-Bahr E, Eckardt L, Breithardt G, et al. Sodium channel gene (SCN5A) mutations in 44 index patients with Brugada syndrome: different incidences in familial and sporadic disease. Hum Mutat 2003;21:651–652. Erratum: Hum Mutat 2005;26:61. [PubMed: 14961552]
9. Remme CA, Wilde AAM. SCN5A overlap syndromes: no end to disease complexity? Europace 2008;10:1253–255. Comment. [PubMed: 18820249]
10. Zimmer T, Surber R. SCN5A channelopathies—an update on mutations and mechanisms. Prog Biophys Mol Biol 2008;98(2–3):120–136. [PubMed: 19027780]
11. Ackerman MJ, Splawski I, Makielski JC, et al. Spectrum and prevalence of cardiac sodium channel variants among black, white, Asian, and Hispanic individuals: implications for arrhythmogenic

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

susceptibility and Brugada/long QT syndrome genetic testing. Heart Rhythm 2004;1:600–607. see Comment. [PubMed: 15851227]

12. Spiegelman JI, Mindrinos MN, Oefner PJ. High-accuracy DNA sequence variation screening by DHPLC. BioTechniques 2000;29:1084–1092. [PubMed: 11084871]
13. Kapa S, Tester DJ, Salisbury BA, et al. Genetic testing for long QT syndrome—distinguishing pathogenic mutations from benign variants. Circulation 2009;120(18):1752–1760. [PubMed: 19841300]
14. den Dunnen JT, Antonarakis SE. Nomenclature for the description of human sequence variations. Hum Genet 2001;109:121–124. [PubMed: 11479744]
15. Murray A, Donger C, Fenske C, et al. Splicing mutations in KCNQ1: a mutation hot spot at codon 344 that produces in frame transcripts. Circulation 1999;100:1077–1084. [PubMed: 10477533]
16. Zhuang Y, Weiner AM. A compensatory base change in U1 snRNA suppresses a 5′ splice site mutation. Cell 1986;46:827–835. [PubMed: 3757028]
17. Rogan PK, Svojanovsky S, Leeder JS. Information theory-based analysis of CYP2C19, CYP2D6 and CYP3A5 splicing mutations. Pharmacogenetics 2003;13:207–218. [PubMed: 12668917]
18. Splawski I, Shen J, Timothy K, Vincent GM, Lehmann MH, Keating MT. Genomic structure of three long QT syndrome genes: KVLQT1, HERG, and KCNE1. Genomics 1998;51:86–97. [PubMed: 9693036]
19. Wang Q, Li Z, Shen J, Keating MT. Genomic organization of the human SCN5A gene encoding the cardiac sodium channel. Genomics 1996;34:9–16. [PubMed: 8661019]
20. Neyroud N, Richard P, Vignier N, et al. Genomic organization of the KCNQ1 K+ channel gene and identification of C-terminal mutations in the long-QT syndrome. Circ Res 1999;84:290–297. [PubMed: 10024302]
21. Wehrens XHT, Rossenbacker T, Jongbloed RJ, et al. A novel mutation L619F in the cardiac Na+ channel SCN5A associated with long-QT syndrome (LQT3): a role for the I-II linker in inactivation gating. Hum Mutat 2003;21:552. [PubMed: 12673799]
22. Ruan Y, Liu N, Bloise R, Napolitano C, Priori SG. Gating properties of SCN5A mutations and the response to mexiletine in long-QT syndrome type 3 patients. Circulation 2007;116:1137–1144. [PubMed: 17698727]
23. Kambouris NG, Nuss HB, Johns DC, Tomaselli GF, Marban E, Balser JR. Phenotypic characterization of a novel long-QT syndrome mutation (R1623Q) in the cardiac sodium channel. Circulation 1998;97:640–644. [PubMed: 9495298]
24. Wei J, Wang DW, Alings M, et al. Congenital long-QT syndrome caused by a novel mutation in a conserved acidic domain of the cardiac Na+ channel. Circulation 1999;99:3165–3171. [PubMed: 10377081]
25. Yang P, Kanki H, Drolet B, et al. Allelic variants in long-QT disease genes in patients with drug-associated torsades de pointes. Circulation 2002;105:1943–1948. [PubMed: 11997281]
26. Chen Q, Kirsch GE, Zhang D, et al. Genetic basis and molecular mechanism for idiopathic ventricular fibrillation. Nature 1998;392:293–296. [PubMed: 9521325]
27. Kapplinger JD, Tester DJ, Salisbury BA, et al. Spectrum and prevalence of mutations from the first 2500 consecutive unrelated patients referred for the *FAMILION*© long QT syndrome genetic test. Heart Rhythm 2009;6:1297–1303. [PubMed: 19716085]
28. Tester DJ, Will ML, Haglund CM, Ackerman MJ. Compendium of cardiac channel mutations in 541 consecutive unrelated patients referred for long QT syndrome genetic testing. Heart Rhythm 2005;2:507–517. [PubMed: 15840476]
29. Meregalli PG, Tan HL, Probst V, et al. Type of SCN5A mutation determines clinical severity and degree of conduction slowing in loss-of-function sodium channelopathies. Heart Rhythm 2009;6:341–348. [PubMed: 19251209]
30. Makita N, Behr E, Shimizu W, et al. The E1784K mutation in SCN5A is associated with mixed clinical phenotype of type 3 long QT syndrome. J Clin Invest 2008;118:2219–2229. [PubMed: 18451998]
31. Bezzina C, Veldkamp MW, van den Berg MP, et al. A single Na^+ channel mutation causing both long-QT and brugada syndromes. Circ Res 1999;85:1206–1213. [PubMed: 10590249]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

32. Probst V, Wilde AAM, Barc J, et al. *SCN5A* Mutations and the role of genetic background in the pathophysiology of brugada syndrome. Circ Cardiovasc Genet. 2009 accepted manuscript, in press.
33. London B, Michalec M, Mehdi H, et al. Mutation in glycerol-3-phosphate dehydrogenase 1 like gene (GPD1-L) decreases cardiac Na+ current and causes inherited arrhythmias. Circulation 2007;116:2260–2268. [PubMed: 17967977]
34. Antzelevitch C. Genetic basis of Brugada syndrome. Heart Rhythm 2007;4:756–757. Comment. Erratum: Heart Rhythm 2007;4:990. [PubMed: 17556198]
35. Watanabe H, Koopman TT, Scouarnec SL, et al. Sodium channel β1 subunit mutations associated with Brugada syndrome and cardiac conduction disease in humans. J Clin Invest 2008;118:2260–2268. [PubMed: 18464934]
36. Delpon E, Cordeiro JM, Nunez L, et al. Functional effects of KCNE3 mutation and its role in the development of Brugada syndrome. Circ Arrhythmia Electrophysiol 2008;1:209–218.
37. Hu S, Barajas-Martinez H, Burashnikove E, et al. A mutation in the β3 subunit of the cardiac sodium channel associated with Brugada ECG phenotype. Circ Cardiovasc Genet 2009;2:270–278. [PubMed: 20031595]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

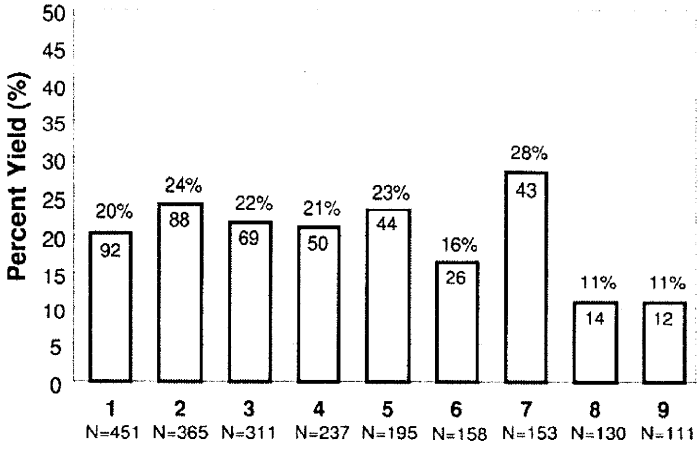

Figure 1.

Mutation detection yield by genetic testing center. Depicted here is a comparison of Brugada syndrome genetic testing for each of the 9 centers ordered according to the total number (N = X) of unrelated patients tested. The number within each column represents the number of genotype positive patients for the respective center. For example, Center 1 analyzed 451 unrelated cases and identified a putative pathogenic mutation in 92 (20%). Center: 1 = Nantes, 2 = Brugada, 3 = AMC, 4 = Paris, 5 = PGxHealth, 6 = MMRL, 7 = UKM, 8 = NCVC, 9 = BCM.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

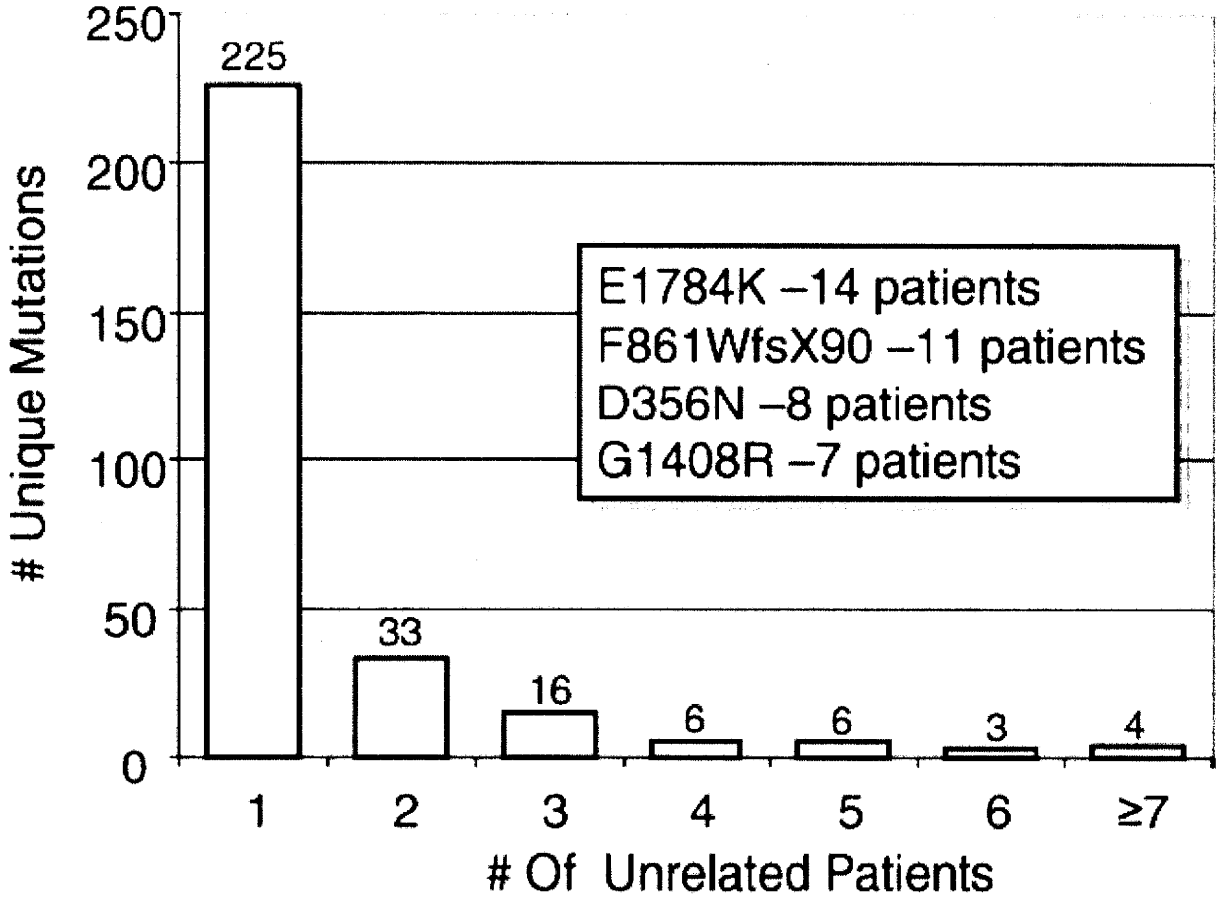

Figure 2.

BrS1-associated mutation frequency distribution. This bar graph summarizes the distribution of specific mutations among unrelated patients. The Y-axis depicts the number of unique BrS1-associated mutations, and the X-axis represents the number of unrelated patients. For example, the first column indicates that there were 226 unique mutations each observed only once. The last column indicates that 4 different BrS1-associated mutations were each seen in ≥7 unrelated patients. The inset shows the 4 most common BrS1-associated mutations identified and the number of specified unrelated patients in whom the mutations were found.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

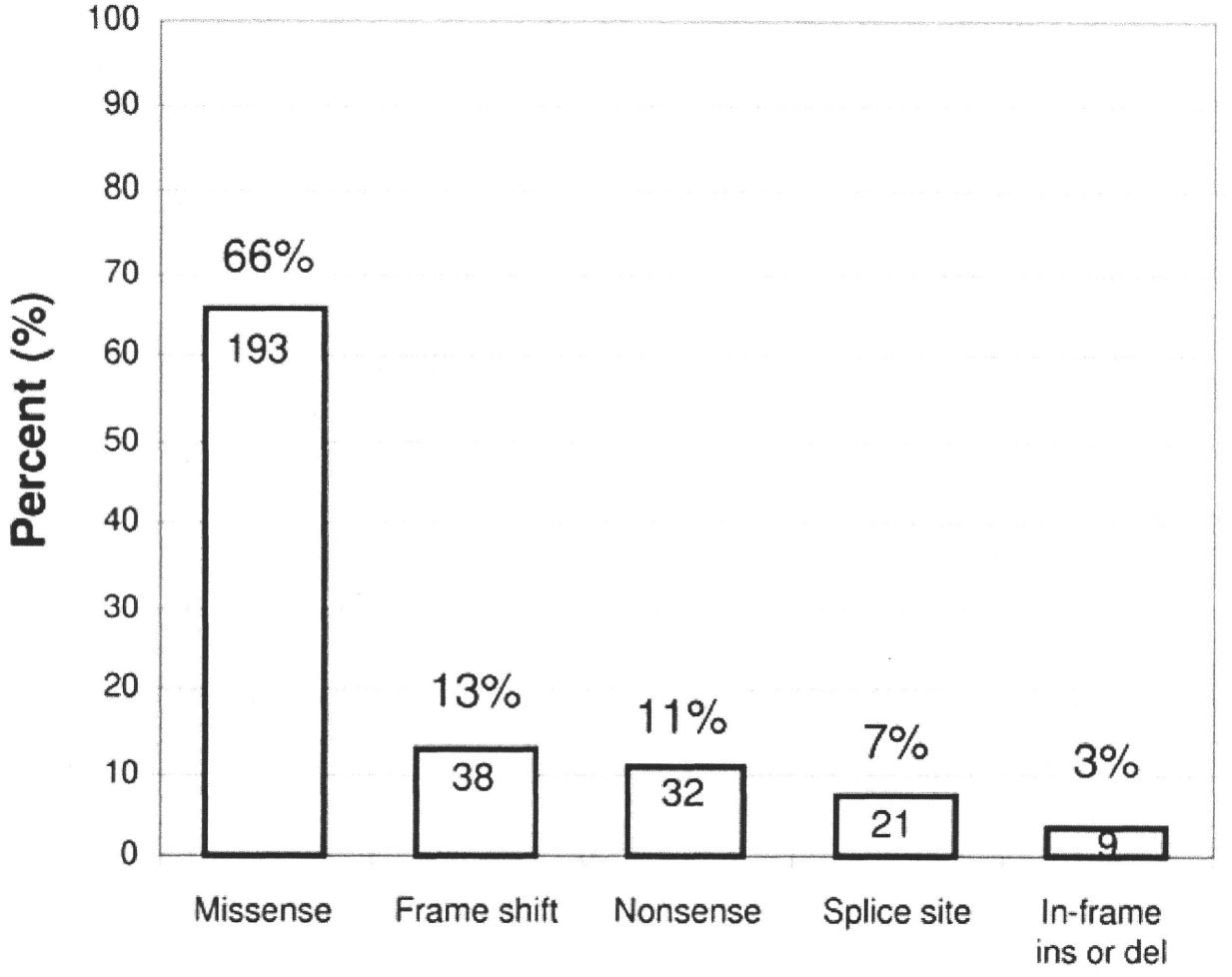

Figure 3.
Summary of *SCN5A* mutation type for BrS1. The distribution of mutation type (missense, frameshift, etc.) is summarized for the possible BrS1-associated *SCN5A* mutations. The number within the column represents the total number of unique mutations for the respective type. For example, there were 193 unique missense mutations identified.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

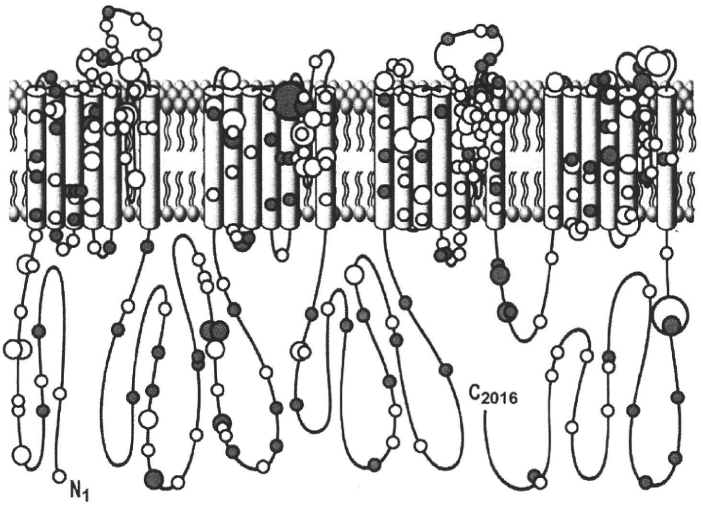

Figure 4.
Channel topology of NaV1.5's pore-forming alpha subunit encoded by *SCN5A* and location of putative BrS1-causing mutations. Missense mutations are indicated by white circles, whereas mutations other than missense (i.e., frameshift, deletions, splice-site, etc.) are depicted as gray circles. In addition, 4 different circle sizes are used, with the smallest circle indicating a mutation seen only once; a medium-sized circle for mutations observed in 2, 3, or 4 unrelated patients; a large circle for mutations observed in 5, 6, 7, 8, or 9 patients; and the largest circle indicating those mutations observed in at least 10 unrelated patients.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

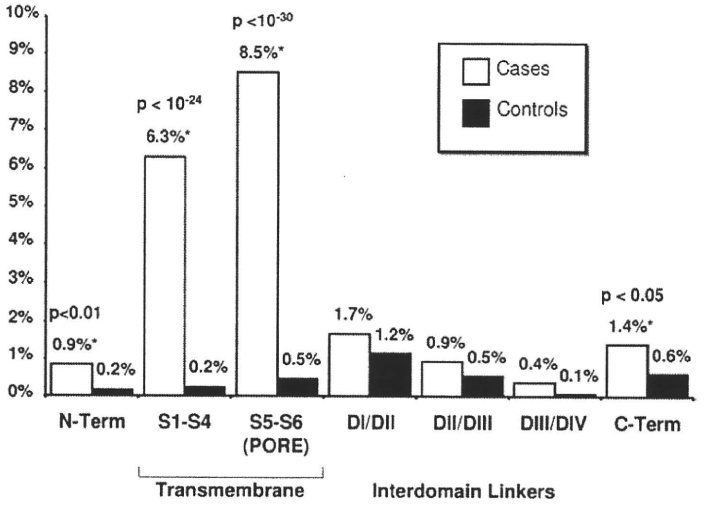

Figure 5.

Yield of missense mutations/rare variants in cases and control subjects by location. A comparison of the yield of rare, missense case mutations/control variants in 2,111 cases versus 1,300 control subjects by protein location. * = $p<0.05$.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Table 1

Demographics and mutation yield for 9 Brugada syndrome genetic testing centers

	1	2	3	4	5	6	7	8	9
Total	451	365	311	237	195	158	153	130	111
Positive	92	88	69	50	44	26	43	14	12
Age (yrs)	47 ± 14	40 ± 11	45 ± 18	43 ± 14	35 ± 18	36 ± 21	45 ± 15	48 ± 10	37 ± 21
Range (yrs)	3 to 70	0 to 77	0 to 82	8 to 80	0 to 76	0 to 69	5 to 65	28 to 64	7 to 78
Yield (%)	20.4	24.1	22.2	21.1	22.6	16.5	28.1	10.8	10.8
Male	357	277	239	193	140	117	106	124	72
Positive	68	67	55	40	31	17	30	13	7
Yield (%)	21.6	24.2	22.2	20.7	23.4	14.5	28.3	10.5	9.7
Female	94	88	72	44	55	41	47	6	39
Positive	23	19	14	10	13	9	13	1	5
Yield (%)	25.5	21.6	19.4	22.7	23.6	22.0	27.7	16.7	12.8

Center: 1 = Nantes, 2 = Brugada, 3 = AMC, 4 = Paris, 5 = PGxHealth, 6 = MMRL, 7 = UKM, 8 = NCVC, 9 = BCM.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Table 2

Control variants found in 2,600 reference alleles

Exon	Nucleotide change	Variant	Mutation type	Location	Number	Ethnicity	Status
2	52 C>T	R18W	Missense	N-terminal	1	O	Rare control
2	100 C>T	R34C	Missense	N-terminal	44	B>H=O>W>A	Polymorphism
2	101 G>A	R34H	Missense	N-terminal	1	B	Rare control
6	647 C>T	S216L	Missense	DI-S3/S4	4	W	Polymorphism
7	856 G>T	A286S	Missense	DI-S5/S6	1	B	Rare control
7	872 A>G	N291S	Missense	DI-S5/S6	1	O	Rare control
7	895 T>A	L299M	Missense	DI-S5/S6	1	B	Rare control
9	1126 C>T	R376C	Missense	DI-S5/S6	1	W	Rare control
11	1340 C>G	A447G	Missense	DI/DII	1	B	Rare control
11	1345 A>G	T449A	Missense	DI/DII	1	O	Rare control
11	1381 T>G	L461V	Missense	DI/DII	2	B	Polymorphism
11	1425 A>C	R475S	Missense	DI/DII	1	B	Rare control
11	1441 C>T	R481W	Missense	DI/DII	6	B>H=O	Polymorphism
12	1571 C>A	S524Y	Missense	DI/DII	18	B>W=H	Polymorphism
12	1673 A>G	H558R	Missense	DI/DII	408	W>B>H>O>A	Polymorphism
12	1703 G>A	R568H	Missense	DI/DII	1	W	Rare control
12	1735 G>A	G579R	Missense	DI/DII	1	W	Rare control
12	1776 C>A	N592K	Missense	DI/DII	1	W	Rare control
12	1787 A>G	D596G	Missense	DI/DII	1	B	Rare control
12	1802 T>C	V601A	Missense	DI/DII	1	W	Rare control
12	1852 C>T	L618F	Missense	DI/DII	1	O	Rare control
13	1913 G>A	G638D	Missense	DI/DII	1	A	Rare control
13	1967 C>T	P656L	Missense	DI/DII	1	B	Rare control
13	2014 G>A	A672T	Missense	DI/DII	1	A	Rare control
14	2066 G>A	R689H	Missense	DI/DII	1	H	Rare control
14	2074 C>A	Q692K	Missense	DI/DII	3	W	Polymorphism
14	2114 C>T	S705F	Missense	DI/DII	1	A	Rare control
16	2770 G>A	V924I	Missense	DII-S6	2	B=O	Polymorphism
17	2924 C>T	R975W	Missense	DII/DIII	1	W	Rare control

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Exon	Nucleotide change	Variant	Mutation type	Location	Number	Ethnicity	Status
17	2957 G>A	R986Q	Missense	DII/DIII	1	B	Rare control
17	3047 C>T	T1016M	Missense	DII/DIII	1	A	Rare control
17	3118 G>A	G1040R	Missense	DII/DIII	1	B	Rare control
18	3245 T>C	V1082A	Missense	DII/DIII	1	B	Rare control
18	3269 C>T	P1090L	Missense	DII/DIII	5	A>W	Polymorphism
18	3292 G>T	V1098L	Missense	DII/DIII	1	A	Rare control
18	3308 C>A	S1103Y	Missense	DII/DIII	31	B>O>H	Polymorphism
18	3319 G>A	E1107K	Missense	DII/DIII	1	A	Rare control
18	3346 C>T	R1116W	Missense	DII/DIII	2	A	Polymorphism
20	3578 G>A	R1193Q	Missense	DII/DIII	12	A>W	Polymorphism
21	3751 G>A	V1251M	Missense	DIII-S2	1	B	Rare control
22	3878 T>C	F1293S	Missense	DIII-S3/S4	2	W	Polymorphism
22	3922 C>T	L1308F	Missense	DIII-S4	3	O>B	Polymorphism
Intron 24	4299 +2 T>A	1433sp	Splice site	DIII-S5/S6	1	W	Rare control
26	4534 C>T	R1512W	Missense	DIII/DIV	1	H	Rare control
28	5360 G>A	S1787N	Missense	C-terminal	3	W	Polymorphism
28	5507 T>C	I1836T	Missense	C-terminal	1	B	Rare control
28	5701 G>A	E1901K	Missense	C-terminal	1	W	Rare control
28	5755 C>T	R1919C	Missense	C-terminal	1	B	Rare control
28	5851 G>T	V1951L	Missense	C-terminal	16	H>A>B=O	Polymorphism
28	5873 G>A	R1958Q	Missense	C-terminal	1	B	Rare control
28	5885 C>T	P1962L	Missense	C-terminal	1	B	Rare control
28	5904 C>G	I1968M	Missense	C-terminal	1	B	Rare control
28	5972 G>A	R1991Q	Missense	C-terminal	1	B	Rare control
28	6010 T>C	F2004L	Missense	C-terminal	7	W>H	Polymorphism
28	6016 C>G	P2006A	Missense	C-terminal	3	W>B	Polymorphism

The > and = symbols represent the relative prevalence of the variant of interest in each corresponding ethnicity.

A = Asian; B = black; H = Hispanic; O = other; and W = white.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Table 3

Brugada syndrome patients with multiple *SCN5A* mutations

Gender	Age at diagnosis (yrs)	Mutation 1	Location	Mutation 2	Location
M	35	N109K	N-terminal	V240M	DI-S4/S5
M	16	A185V	DI-S2/S3	A226V	DI-S4
M	44	611+1 G>A	DI-S3	V300I	DI-S5/S6
M	26	T220I	DI-S4	E439K	DI-DII
M	21	934+4 C>T	DI-S5/S6	G1642E	DIV-S4
M	51	P336L	DI-S5/S6	I1660V	DIV-S5
M	40	L619F	DI-DII	Q1383X	DII-S5/S6
M	49	T632M	DI-DII	M764R	DII-S2
M	2	Q646RfsX5	DI-DII	D1243N	DIII-S2
M	24	A647D	DI-DII	P1332L	DIII-S4/S5
M	7	G752R	DII-S2	K1872N	C-terminal
M	41	E1053K	DII-DIII	R1583C	DIV-S2/S3
M	23	R1232W	DIII-S1/S2	T1620M	DIV-S3/S4

M = male.