Systematic Re-evaluation of SCN5A Variants Associated with Brugada Syndrome

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Systematic Re-evaluation of SCN5A Variants Associated with Brugada Syndrome

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Abstract

**Background:** A large number of SCN5A variants have been reported to underlie Brugada syndrome (BrS). However, the evidence supporting individual variants is highly heterogeneous.

**Objective:** We systematically re-evaluated all SCN5A variants reported in BrS using the 2015 ACMG-AMP guidelines.

**Methods:** A Pubmed/Embase search was performed to identify all reported SCN5A variants in BrS. Standardized bioinformatic re-analysis (SIFT, PolyPhen, Mutation Taster, Mutation assessor, FATHMM, GERP, PhyloP, and SiPhy) and re-evaluation of frequency in the gnomAD database was performed. 14 ACMG-AMP rules were deemed applicable for SCN5A variant analysis.

**Results:** 480 unique SCN5A variants were identified, the majority of which 425 (88%) were coding variants. 156/425 (37%) variants were classified as pathogenic/likely pathogenic. 258 (60%) were classified as variants of uncertain significance, while a further 11 (3%) were classified as benign/likely benign. When considering the subset of variants that were considered ‘null’ variants separately, 95% fulfilled criteria for pathogenicity/likely pathogenicity. On the other hand, only 17% of missense variants fulfilled criteria for pathogenicity/likely pathogenicity. Importantly however, only 25% of missense variants had available functional data, which was a major score driver for pathogenic classification.

**Conclusion:** Based on contemporary ACMG-AMP guidelines, only a minority of ion channel variants implicated in BrS fulfil criteria for pathogenicity or likely pathogenicity.

**Keywords:** Brugada, Genetics, Arrhythmia, SCN5A, sodium channel

**Introduction**

Brugada syndrome (BrS) is a genetic arrhythmia syndrome characterized by spontaneous or drug-induced J point elevation in the right precordial ECG leads and is associated with a risk of malignant ventricular arrhythmias and sudden cardiac death (SCD). Over the past two decades, significant progress has been made in elucidating the genetic basis of BrS. According to current estimates, causative mutations are identified in a third of patients with BrS,\(^1\)\(^-\)\(^3\) the vast majority of which have been identified in the SCN5A gene.\(^4\) Based on recent evidence, of the 21 genes implicated in BrS, only SCN5A has definitive evidence linking it to a BrS phenotype.\(^5\)

Interpretation of genetic data in patients with BrS remains complex. With the emergence of more refined strategies for variant classification and rapidly emerging data on genetic variation in normal populations, periodic re-evaluation...
of genetic variants underlying BrS is an important consideration. Re-evaluation of
genetic variants in *SCN5A* is particularly relevant as the gene has a high
background rate of rare variation.\textsuperscript{6,7} Furthermore, there is a high degree of
variation in the strategies used for analysis of previously reported BrS variants.

In the present study, we re-assessed pathogenicity of all *SCN5A* variants
previously reported in BrS using a modified version of the 2015 ACMG-AMP
guidelines.\textsuperscript{8} We used a standardized approach for variant assessment using the
gnomAD population database and *in silico* variant prediction tools.

Methods

2.1 Systematic literature search

A Pubmed/Embase search was performed on 12\textsuperscript{th} January 2017 using the search
terms ‘Brugada’. Based on the association between *SCN5A* variants and other
overlapping phenotypes (conduction abnormalities, short-QT syndrome, long-QT
syndrome), additional searches were performed using the terms ‘*SCN5A* mutation’
or ‘*SCN5A* variant’. Abstracts for all identified manuscripts were reviewed. All
studies that included cases/cohorts of BrS patients and all studies involving
functional analysis of genetic variants were reviewed in full. Only English
language articles of the following categories were included in final analysis; 1) original
studies/case reports identifying genetic variants, 2) studies investigating
the functional significance of genetic variants, and 3) compendia of genetic
variants underlying BrS.

Where available, ECGs from original manuscripts were reviewed and a diagnosis
of BrS reconfirmed by two independent investigators. A diagnosis of BrS was
based on current consensus guidelines.\textsuperscript{9}

2.2 Analysis of pathogenicity

The strategy for variant analysis was based on the 2015 ACMG-AMP guidelines.
A detailed breakdown of the scoring system is included in \textit{Tables 1 and 2}. Of the
27 ACMG-AMP rules, 13 were deemed not to be applicable. Details on the
rationale for excluding these criteria are included in the Supplemental text.

\textbf{2.2.1 Analysis of segregation}

Criteria for variant segregation (PP1) included: 1) presence in \geq 2 family members
with BrS within the same pedigree or 2) presence in 1 family member with BrS
and \geq 1 family members with a related phenotype (conduction abnormality, short-
QT syndrome, long-QT syndrome - in the same pedigree with a complex
overlapping phenotype) or 3) reported in an isolated BrS case but also reported in a
\textit{separate} pedigree (\geq 2 family members) with BrS-related phenotypes (in the
absence of BrS in that pedigree). Evidence from segregation analysis was
upgraded from ‘supporting’ to ‘moderate’ in the presence of linkage data with a
LOD score of \geq 3. The presence of affected non-carriers was considered as strong
evidence of benign status (BS4). The ACMG-AMP recommendation for
demonstration of segregation in more than one family was waived as *SCN5A* is an
established BrS gene.

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2.2.2 Analysis of functional data

Cellular electrophysiology studies demonstrating a loss-of-function effect were considered as strong evidence (PS3). Functional evidence was considered insufficient for PS3 if cellular electrophysiology studies demonstrated a <50% reduction in peak $I_{Na}$ without significant gating abnormalities predicted to decrease current under physiological conditions. Functional data was also excluded in the presence of conflicting evidence between studies. Functional studies demonstrating no effect on peak $I_{Na}$ (in the absence of gating abnormalities) were considered as supportive evidence of benign status (BS3).

2.2.3 Analysis of variant frequency

Variant frequency was analyzed in the gnomAD browser, which spans ~123,000 exomes (67,101 European; 16,248 Latino; 15,391 South Asian; 7,652 African; 8,624 East Asian; 4,925 Ashkenazi Jewish; http://gnomad. broadinstitute.org/). Variant frequency was classified as ‘ultra rare’, ‘rare’ and ‘common’ using a similar strategy to a recent report by Ackerman and colleagues. The prevalence of BrS due to SCN5A variants is predicted to be 1/10,000 (BrS prevalence 1/2000 with estimated prevalence of SCN5A variants in BrS of 1/5). Therefore, ~12 individuals in gnomAD would be predicted to have BrS due to SCN5A variants. Given that the majority of variants reported to underlie BrS are ‘private’ mutations, the likelihood that any given variant is present in ≥5 of the estimated 12 BrS patients with SCN5A causative variants is low. In order to correct for differences in SCN5A variant frequency between different ethnicities, we applied a more rigorous approach to analysis of variant frequency by calculating ethnicity-specific variant frequency. A threshold of ≥3 variant carriers in at least one ethnic group resulted in a re-classification of a variant as ‘common’. Overall, variants were classified as: 1) ultra rare (absent in overall gnomAD; PM2), 2) rare (1-4 individuals in global gnomAD and present in <3 individuals for any one ethnicity), 3) common (present in ≥5 individuals in global gnomAD and/or ≥3 individuals in at least one ethnic group; BS1), 4) very common (MAF >5%; BA1)

2.2.4 Variant enrichment in BrS cases

The gnomAD population was used as a proxy for healthy controls. Only ‘ultra-rare’ variants in BrS cohorts (absent from global gnomAD cohort) were considered for enrichment analysis. In order to minimize the risk of duplicate counting (due to authorship overlap and inter-laboratory collaborations), for those variants that were reported by the same authors in multiple studies, only the study reporting the maximum number of variants was considered. The presence of the variant in ≥5 BrS cases was considered as strong evidence (PS4).

2.2.5 Computational evidence

In silico analysis was performed with 5 protein-level prediction tools; SIFT, PolyPhen, Mutation Taster, Mutation assessor, FATHMM and 3 conservation metrics; GERP++, PhyloP conservation and SiPhy (thresholds for conservation for GERP++, PhyloP and SiPhy were 4.4, 1.6 and 12.17, respectively). A predicted pathogenic effect with ≥6 in silico tools was

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considered as supporting evidence (PP3) while ≤2 tools was considered as supporting evidence of benign status (BP4).

2.2.6 Location in critical functional domain

The universal protein resource (www.uniprot.org) was used to classify variant location within the major SCN5A topology regions: N-terminus (1-131), C-terminus (1772-1316), transmembrane/pore-forming (D1, 132-410; D2, 718-938; D3, 1208-1466; D4, 1530-1771), interdomain linker (D1/D2, 411-717; D2/D3, 939-1206; D3/D4, 1467-1529). A missense variant located in a transmembrane spanning or pore-forming domain was considered as moderate evidence (PM1) based on previous studies reporting these as critical locations. Null variants were not scored according to this criterion.

2.3 Statistics

Analysis of the relative contribution of each score component to the final variant classification was performed using a multiple logistic regression (SigmaPlot v11.0). Each score component was included as an independent variable. Regression coefficients were calculated as the natural logarithm of odds ratios. Statistical significance was taken as p<0.05 and if the 95% confidence intervals for regression coefficients did not cross 0.

Results

3.1 Identification of studies

A flow chart demonstrating criteria for study selection is included in Figure 1. An initial search identified 4122 publications. Following systematic abstract review, 547 publications were retained for full text review. Following sequential filtering, 212 publications focusing on genetic analysis in BrS and/or conduction disease were included for final analysis (Supplemental table 1).

3.2 Summary of variants

536 unique SCN5A variants were identified to underlie BrS and/or conduction disease. In the present study, we focused only on variants that resulted in a BrS phenotype in at least one variant carrier (480 SCN5A variants fulfilled this criterion). SCN5A variants that have been reported to result in conduction abnormalities only (in the absence of a BrS phenotype) were not analyzed using the ACMG-AMP guidelines (a list of these variants is included in Supplemental table 2).

Of the aforementioned 480 SCN5A variants resulting in a BrS phenotype, 55 non-coding variants were excluded from further analysis (36 intronic splice site variants and 19 promoter region variants). Of the remaining 425 coding variants (303 missense, 53 frameshift, 48 truncating, 12 deletions, 7 insertions and 2 duplications), 104 (24%) were classified as ‘null variants’ according to the ACMG-AMP definitions outlined in Table 1 (53 frameshift variants; 48 truncating variants; 3 insertion variants).
ECGs were available for review for 102/425 (24%) coding variants. A diagnosis of BrS was reconfirmed in 98 of these 102 cases (96%) following independent ECG review (by two investigators). For the 4 (4%) remaining variants (S835L, I848SfsX33, R965C, and A1428S), a diagnosis of BrS could not be made based on the ECG provided in the manuscript. For the remaining 323 (76%) there were no details on the ECG (descriptive or ECG traces). 159/425 (37%) variants had a detailed clinical history of the proband/pedigree. 65/159 (41%) were reported to be associated with ventricular arrhythmia (sustained ventricular tachycardia or fibrillation) or aborted SCD. 69/159 (44%) were associated with syncope. 51/159 (32%) were associated with a family history of SCD.

Segregation data were available for 113/425 (27%) coding variants (Supplemental table 3). 80/113 (71%) were reported to co-segregate (46/80 BrS only; 28/80 BrS and complex phenotypes in the same pedigree; 6/80 segregation with complex phenotypes and isolated BrS proband reported independently). Overall, a median of 3 phenotype-positive variant carriers was identified per pedigree (IQR 3). 10/113 (9%) variants were identified in pedigrees with genotype-negative and phenotype-positive family members. 3/113 (3%) were de novo variants. One variant was upgraded to ‘moderate’ evidence based on a LOD score of >3.24,25

Functional data was available for 114/425 (27%) coding variants (Supplemental table 4). Functional assessment was performed using patch-clamp electrophysiology, although specific techniques varied. 41/114 (36%) variants were co-expressed with wild type SCN5A α-subunits and 69/114 (61%) were co-expressed with β-subunits. 80/114 (70%) variants demonstrated a significant loss-of-function effect.

301/425 (71%) coding variants were classified as ‘ultra rare’ (absent from global gnomAD population). When analyzing variant frequency in the global gnomAD database (~123,000 exomes), 62 (14.5%) variants were classified as ‘rare’ (present in 1-4 individuals) and 62 (14.5%) were classified as ‘common’ (present in ≥5 individuals). Based on the ethnicity-adjusted analysis, 52/425 (12%) were classified as rare, and 72/425 (17%) were classified as common (Supplemental table 5). Overall therefore, 10/425 (2%) variants were reclassified based on ethnicity-specific analysis. 9/425 (2%) variants were not only absent in the global gnomAD population but were also statistically overrepresented in BrS cases.

Of the 303 analyzed SCN5A variants, 243 (80%) had a predicted pathogenic effect with ≥6 in silico tools, and 26 (9%) had a predicted pathogenic effect with ≤2 in silico tools (Supplemental table 6).

3.3 ACMG-AMP variant scoring

Overall, based on a modified version of the ACMG-AMP guidelines, only 37% of SCN5A variants fulfilled criteria for a pathogenic or likely pathogenic classification (pathogenic, n=39; likely pathogenic, n=117). 3% were classified as benign or likely benign (benign, n=3; likely benign, n=8). 258/425 (60%) were classified as VUS. Variant scores are summarized in Figure 2 and Supplemental table 7.
Based on multiple logistic regression analysis, the strongest score drivers for a pathogenic/likely pathogenic classification were: ‘null variant’ status (P1; regression coefficient 10.0) and functional evidence (P3; regression coefficient 6.4). Other score drivers included absence in gnomAD (P6; regression coefficient 6.2), segregation (P8; regression coefficient 4.6), and bioinformatic prediction (P9; regression coefficient 2.2). Confidence intervals for de novo mutation (P2), frequently reported unique variants (P4), critical location (P5), and location association (P7) crossed zero and these criteria were therefore not deemed to be major score drivers (Figure 3).

When considering the subset of null variants separately, the majority (99/104 [95%]) fulfilled criteria for pathogenicity or likely pathogenicity (50/53 [94%] frameshift variants; 46/48 [94%] truncating variants; 3/3 [100%] insertion variants). For the majority of these variants, the additional determinant of variant pathogenicity was ‘ultra rare’ status (99% variants; moderate evidence [P6]). Other score drivers contributing to pathogenic/likely pathogenic classification for null variants included functional data (15%; P3, strong evidence) and variant segregation (12%; P8, supporting evidence).

When considering the subset of missense variants separately, only 51/303 (17%) fulfilled criteria for pathogenicity/likely pathogenicity. The classification of pathogenicity/likely pathogenicity was determined by functional evidence (78%, [P3]), agreement between ≥6 bioinformatic tools (96%, [P9]), ‘ultra rare’ status (98%, [P6]), and variant segregation (73%, [P8]). 11/303 (4%) missense variants were classified as likely benign or benign. Benign/likely benign status was largely driven by variant frequency in gnomAD [B2] (100%), with additional determinants including functional studies demonstrating no damaging effect (29%, [B3]) and ≤2 bioinformatic prediction tools suggesting pathogenic effect (86%, [B5]).

We investigated whether pathogenic variants clustered to a specific region of the protein (Figure 2). The 5 null variants that were classified as VUS were evenly distributed across the major topology regions. Missense variants classified as pathogenic/likely pathogenic were more likely to be found in transmembrane regions of the protein, in part due to criterion [P6] (transmembrane n=39, C-terminus n=5, interdomain linker n=4, N-terminus n=3). Interestingly, all 11 variants deemed benign or likely benign localized to intracytoplasmic regions of the protein (N-terminus n=2, C-terminus n=3, cytoplasmic regions of transmembrane-spanning domains n=6).

Comparison with an SCN5A-specific scoring system

We compared the our ACMG-AMP classifications of missense variants to those derived from an classification system designed by Kapplinger and colleagues, which is largely based on analysis of protein topology and in silico prediction.\textsuperscript{26} Of the 171 unique variants reported in the Kapplinger study, only 95 (56%) had consistent classifications with our ACMG-AMP classifications (Supplemental table 7). The majority (82%) of the inconsistent classifications involved a
downgrade from a ‘Probably pathogenic’ classification with the Kapplinger system to a ‘VUS’ classification with the ACMG-AMP classification.

Discussion

In the present study, we demonstrate that based on contemporary ACMG-AMP guidelines, only 37% of \textit{SCN5A} variants previously implicated in BrS fulfil criteria for pathogenicity or likely pathogenicity, the majority of which are null variants. Overall, most variants, and the vast majority of missense variants, were classified as VUS. Only 3% of all variants were classified as benign or likely benign.

The presence of preexisting studies implicating a genetic variant in disease has an important influence whether the variant is deemed pathogenic. However, one of the major challenges to interpretation of preexisting evidence, particularly in the context of conditions such as BrS, is the significant heterogeneity in the approaches used for variant analysis. Important sources of heterogeneity include incomplete/absent functional and segregation data, variations in approaches used for functional and computational analysis, and variations in the sizes of the cohorts used to determine variant prevalence in the general population. In the present study, we addressed some of the sources of heterogeneity by applying standardized \textit{in silico} analysis and analysis of variant frequency. However, the absence of functional and segregation data for a large number of studies remains an important confounding factor. This limitation is particularly relevant when considering the fact that functional evidence was the major score driver for pathogenicity/likely pathogenicity for missense variants.

It is important to emphasize that the ACMG-AMP guidelines are primarily designed for analysis of monogenic traits and increasing evidence is emerging to suggest that BrS is an oligogenic disorder with complex association. In addition to studies reporting compound mutations in BrS patients, genome-wide association studies have reported that common variants influence susceptibility to BrS.\textsuperscript{27-29} Further, pedigree-based studies have reported a low disease penetrance among \textit{SCN5A} variant carriers.\textsuperscript{30} Indeed, in a study involving large BrS pedigrees, Probst et al identified multiple \textit{SCN5A} variant-negative and phenotype-positive family members, suggesting that \textit{SCN5A} variants are not sufficient to cause BrS and genetic background is a powerful modulator of disease expression.\textsuperscript{31} The challenges relating to application of ACMG-AMP guidelines to analysis of oligogenic traits are highlighted by classification of variants such as E161K. Despite extensive evidence supporting pathogenicity, E161K was classified as a VUS on the basis of genotype-negative and phenotype-positive family members in BrS pedigrees. Overall, using the ACMG-AMP guidelines to elucidate the complex genetic architecture underlying BrS is associated with important potential limitations and the variant classification in the present study should be interpreted with caution. Despite these limitations however, we believe that selected ACMG-AMP criteria for variant analysis remain relevant for analysis of BrS variants with large effect sizes and in the absence of validated scoring systems for oligogenic traits, these criteria provide valuable information on potential pathogenicity.\textsuperscript{26-28}

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The ACMG-AMP framework is designed for universal applicability and the use of the criteria for BrS is associated with important potential limitations. More specifically, the lack of granularity for a number of guidelines is an important contributor to variations in classification. This point is underscored by the differences in approach to variant classification between the present study as a recent report by Kroncke and colleagues. They used a subset of ACMG-AMP criteria to analyze pathogenicity of SCN5A variants. While their study involved a combination of BrS and LQTS (with two-thirds of variants associated with BrS), as compared to the present study, a higher proportion of SCN5A variants (47%) fulfilled criteria for likely pathogenicity. There are a number of differences in their approach that may account for differences in classification. First, they applied four of the ACMG-AMP criteria in their study, while we applied 14 criteria. Second, they deemed all missense SCN5A as fulfilling criterion PP2 (missense variant in a gene with a low rate of benign missense variation and missense variants are a common mechanism of disease). Based on a reported background SCN5A variation rate of between 2-5%, we deemed this criterion not to be applicable. Third, their criterion for enrichment (PS4; prevalence of variant in affected individuals significantly increased compared with controls) was set to >20% of carriers presenting with either BrS or LQT3. In the present study, we set a specific enrichment threshold which we believe aligns more closely with ACMG-AMP recommendations. Finally, while their study involved two bioinformatics tools our study involved five.

Kapplinger and colleagues recently developed an enhanced scoring system for SCN5A variants underlying BrS. Interestingly, we demonstrated a relatively high degree of inconsistency between our ACMG-AMP guideline-based approach and scores generated using the aforementioned enhanced scoring system. ACMG-AMP guideline-based scoring ‘downgraded’ close to a third of variants to a VUS status. There are a number of explanations for the differences between the scoring systems. First, while the Kapplinger scoring is largely driven by protein topology and in silico prediction, the ACMG-AMP guidelines are less weighted towards these criteria. Second, the ACMG-AMP guidelines are more stringent in terms of the number of criteria necessary for pathogenic classification. Third, the ACMG-AMP classifications are based on more diverse criteria. These inconsistencies underscore the point that because the ACMG-AMP guidelines are designed for general applicability, some rules are potentially overly conservative.

As discussed above, the presence of functional data is a major score driver for missense variants. However, analysis of functional data for variant scoring represents a challenge for a number of reasons. First, binary characterization of functional evidence is associated with limitations. In an elegant recent study Kroncke and colleagues demonstrated a continuous relationship between functional perturbations and penetrance of BrS. Second, among the subset of variants with available functional data, we observed significant heterogeneity in the level of functional evidence, with some studies reporting \( I_{\text{Na}} \) abolition and others demonstrating more moderate effects. Third, significant confounding factors remain as the methods used to assess \( I_{\text{Na}} \) channel function varied considerably. Accurate assessment of \( I_{\text{Na}} \) channel function is reliant on channel expression in mammalian cell lines, co-expression of mutant channels with \( \beta \)-subunits and wild-type channel proteins, and recording currents at physiological conditions.
temperatures. Only a minority of $I_{Na}$ functional studies took all of these considerations into account. These findings highlight the challenges of accurate interpretation of functional data in the context of variant scoring. Finally, there is a paucity of data on how perturbations in ion channel function reported from cellular studies relate to arrhythmia susceptibility on an organ scale.

4.1 Limitations

There are a number of potential limitations associated with our analysis. First, variant interpretation is subject to inherent inter-laboratory variation in interpretation. In the present study, we did not test for inter- or intra-laboratory reproducibility of variant classification. Second, phenotypic data, and in particular ECG data, was only available for a quarter of variants. The strength of the phenotypic data plays an important role in the interpretation of genotypic findings. Finally, there are multiple loss-of-function $SCN5A$ variants that lead to phenotypes other than BrS. While we have included a list of these variants, scoring was restricted to variants where at least one carrier presented with BrS.

4.2 Conclusions

Following systematic and standardized variant analysis, a high proportion of variants implicated in BrS did not fulfil criteria for pathogenicity according to modified ACMG-AMP guidelines. However, the use of the guidelines for analysis of BrS variants is associated with important potential limitations. These findings underscore the importance of careful interpretation of pre-existing evidence in the context of variant scoring.

References


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Table 1. 2015 ACMG-AMP rules used for assessment of variant pathogenicity.

<table>
<thead>
<tr>
<th>[P1] Null variant</th>
<th>Strength of evidence</th>
<th>Original ACMG category</th>
<th>Modified criteria for scoring variant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Very strong</td>
<td>PVS1</td>
<td>Null SCN5A variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion)</td>
</tr>
<tr>
<td>[P2] De novo variant</td>
<td>Strong</td>
<td>PS1</td>
<td>Same amino acid change as a previously established pathogenic variant (with different nucleotide change)</td>
</tr>
<tr>
<td>[P3] Functional implications of variant</td>
<td>Strong</td>
<td>PS3</td>
<td>De novo variant (both maternity and paternity confirmed) in a patient with BrS and no family history</td>
</tr>
<tr>
<td></td>
<td>Strong</td>
<td>Functional studies with either: (1) a ≥50% decrease in peak $I_{Na}$, (2) significant gating abnormality predicted to decrease current (activation shift +10mV OR inactivation shift -10mV OR sum ≥10mV).</td>
<td></td>
</tr>
<tr>
<td>[P4] Frequently reported unique variant</td>
<td>Strong</td>
<td>PS4</td>
<td>‘Ultra rare’ variant (absent in gnomAD) reported in &gt;5 unrelated individuals with BrS and the variant is absent from the general population. Prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls</td>
</tr>
<tr>
<td>[P5] Critical location of variant</td>
<td>Moderate</td>
<td>PM1</td>
<td>Located in a well-established functional domain (transmembrane or pore-forming domain) of the ion channel. Does not apply if meeting [P1] null variant.</td>
</tr>
<tr>
<td>---------------------------------</td>
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<td>----------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>[P6] Unique variant</td>
<td>Moderate</td>
<td>PM2</td>
<td>‘Ultra rare’ (absent in gnomAD), For recessive disorders, detected in <em>trans</em> with a pathogenic variant</td>
</tr>
<tr>
<td>[P7] Location of variant associated with pathogenicity</td>
<td>Moderate</td>
<td>PM3</td>
<td>Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before</td>
</tr>
<tr>
<td>[P8] Segregation of BrS and variant</td>
<td>Supporting</td>
<td>PP1</td>
<td>Co-segregation with BrS and/or complex phenotypes in multiple affected family members</td>
</tr>
<tr>
<td>[P9] Predicted implications of variant</td>
<td>Supporting</td>
<td>PP2</td>
<td>Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease</td>
</tr>
<tr>
<td></td>
<td>Supporting</td>
<td>PP3</td>
<td>Agreement between $\geq 6$ bioinformatic prediction tools that the variant is pathogenic</td>
</tr>
</tbody>
</table>
Supporting PP4 Patient’s phenotype or family history is highly specific for a disease with a single genetic etiology

Supporting PP5 Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation

<table>
<thead>
<tr>
<th>Modified ACMG category for BrS</th>
<th>Strength of evidence</th>
<th>Original ACMG category</th>
<th>Modified criteria for scoring variant</th>
</tr>
</thead>
<tbody>
<tr>
<td>[B1] Very high frequency in control population</td>
<td>Stand alone</td>
<td>BA1</td>
<td>Allele frequency (MAF) is &gt;5% in gnomAD</td>
</tr>
<tr>
<td>[B2] Higher frequency in control population</td>
<td>Strong</td>
<td>BS1</td>
<td>‘Common’ (present in ≥5 individuals in global gnomAD cohort)</td>
</tr>
<tr>
<td>[B3] No functional implication of</td>
<td>Strong</td>
<td>BS3</td>
<td>Well-established in vitro functional studies show no damaging effect on peak $I_{Na}$</td>
</tr>
<tr>
<td>variant</td>
<td>Supporting</td>
<td>BP1</td>
<td>Missense variant in a gene for which primarily truncating variants are known to cause disease</td>
</tr>
<tr>
<td>---------</td>
<td>------------</td>
<td>-----</td>
<td>-------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>[B4] Absence of segregation</td>
<td>Strong</td>
<td>BS4</td>
<td>Absence of segregation of variant evidenced by presence of one or more family members who are genotype-negative phenotype-positive for BrS</td>
</tr>
<tr>
<td>Supporting</td>
<td>BP2</td>
<td>Observed in <em>trans</em> with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in <em>cis</em> with a pathogenic variant in any inheritance pattern</td>
<td></td>
</tr>
<tr>
<td>Supporting</td>
<td>BP3</td>
<td>In-frame deletions/insertions in a repetitive region without a known function</td>
<td></td>
</tr>
<tr>
<td>[B5] Predicted implications of the variant</td>
<td>Supporting</td>
<td>BP4</td>
<td>Agreement between ≤2 bioinformatic prediction tools that the variant is pathogenic</td>
</tr>
<tr>
<td>Supporting</td>
<td>BP5</td>
<td>Variant found in a case with an alternate molecular basis for disease</td>
<td></td>
</tr>
<tr>
<td>Supporting</td>
<td>BP6</td>
<td>Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation</td>
<td></td>
</tr>
</tbody>
</table>
Supporting BP7

<table>
<thead>
<tr>
<th>Variant classification</th>
<th>Rules</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pathogenic</strong></td>
<td></td>
</tr>
<tr>
<td>1 very strong</td>
<td>and one of:</td>
</tr>
<tr>
<td>a) ≥1 strong</td>
<td>(adapted from the ACMG-AMP guidelines).</td>
</tr>
<tr>
<td>b) ≥2 moderate</td>
<td></td>
</tr>
<tr>
<td>c) 1 moderate and 1 supporting</td>
<td></td>
</tr>
<tr>
<td>d) 2 supporting</td>
<td></td>
</tr>
<tr>
<td>≥2 strong</td>
<td></td>
</tr>
<tr>
<td>1 strong</td>
<td>and one of:</td>
</tr>
<tr>
<td>a) ≥3 moderate</td>
<td></td>
</tr>
<tr>
<td>b) 2 moderate and 2 supporting</td>
<td></td>
</tr>
<tr>
<td>Likely Pathogenic</td>
<td>1 very strong and 1 moderate</td>
</tr>
<tr>
<td>-------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td></td>
<td>1 strong and a) 1-2 moderate</td>
</tr>
<tr>
<td></td>
<td>b) 2 supporting</td>
</tr>
<tr>
<td></td>
<td>≥3 moderate</td>
</tr>
<tr>
<td></td>
<td>2 moderate and 2 supporting</td>
</tr>
<tr>
<td>Likely Benign</td>
<td>1 strong and 1 supporting</td>
</tr>
<tr>
<td>Benign</td>
<td>1 stand alone</td>
</tr>
<tr>
<td></td>
<td>≥2 strong</td>
</tr>
<tr>
<td>Variant of unknown significance</td>
<td>a) any above criteria not met</td>
</tr>
<tr>
<td></td>
<td>b) contradictory combination of pathogenic and benign criteria</td>
</tr>
</tbody>
</table>

Figure 1. Flow diagram demonstrating filtering and selection of studies.
Figure 2. Location of variants in SCN5A gene. Each filled circle represents a variant at a specific region of the protein with colour corresponding to likelihood of pathogenicity. Circles with crosses represent premature protein truncation while circles flanked with arrows represent frameshift mutations.

Figure 3. Score drivers. Logistic regression of each score component determining whether a variant is pathogenic/likely pathogenic vs. benign/likely benign/VUS expressed as natural logarithm of odds ratio. Error bars represent 95% confidence intervals, +/- indicate extension of error bars beyond graph axes. Numerals represent number of times this criterion was met.
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