**LACK OF GENOTYPE-PHENOTYPE CORRELATION IN BRUGADA SYNDROME AND SUDDEN ARRHYTHMIC DEATH SYNDROME FAMILIES WITH REPORTED PATHOGENIC *SCN1B* VARIANTS**

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**ABSTRACT**

**Background:**

There is limited evidence that Brugada Syndrome (BrS) is due to *SCN1B* variants (BrS5). This gene may be inappropriately included in routine genetic testing panels for BrS or sudden arrhythmic death syndrome (SADS).

**Objective**:

We sought to characterize genotype-phenotype correlation in BrS and SADS families with reportedly pathogenic *SCN1B* variants and to review their pathogenicity.

**Methods**:

BrS and SADS families were assessed from six inherited arrhythmia centers worldwide and a comprehensive literature review performed. Clinical characteristics including relevant history, electrocardiogram (ECG), and drug provocation testing were studied. *SCN1B* genetic results were reclassified using American College of Medical Genetics criteria.

**Results**:

A total of 23 *SCN1B* genotype positive individuals were identified from 8 families. Four (17%) probands experienced ventricular fibrillation or sudden cardiac death (SCD) at the time of presentation. All family members were free from syncope or ventricular arrhythmias. Only 2/23 (9%) genotype positive individuals demonstrated a spontaneous BrS ECG pattern. Drug challenge testing for BrS in 87% (13/15) was negative. There was no difference in PR (161±7 vs 165±9ms; p=0.83), QRS (101±6 vs 89±5ms; p=0.35) or QTc (414±35 vs 405±8ms; p=0.7) intervals between genotype positive and negative family members. The overall frequency of previously implicated *SCN1B* variants in gnomAD browser is 0.004% exceeding the estimated prevalence of BrS due to *SCN1B* (0.0005%), including 15/23 (65%) individuals who had the p.Trp179X variant.

**Conclusions**:

The lack of genotype-phenotype concordance amongst families, combined with the high frequency of previously reported mutations in the gnomAD browser suggests that *SCN1B* is not a monogenic cause for BrS or SADS.

*(*249words*)*

Key words**:** Brugada syndrome, *SCN1B*, SADS, Sudden Cardiac Death, genotype phenotype correlation, reclassification

**INTRODUCTION**

Brugada Syndrome (BrS) is an inherited arrhythmia syndrome characterized by coved-shaped ST elevation in the right precordial leads on 12-lead electrocardiogram, with patients at risk of sudden cardiac death (SCD) due to ventricular fibrillation.1-3 The prevalence of BrS in the West is estimated at 1:2000 (0.05%). The most common genetic abnormality associated with BrS are loss of function mutations in *SCN5A*, accounting for approximately 20% of cases.4 There are currently a total of 23 genes implicated in BrS (BrS 1-23), however few are based on strong linkage studies and most are rare.4-6 The gene encoding the ß-1 subunit of the sodium channel, *SCN1B* is currently known as BrS5 and the association with BrS and conduction disease was first reported in 2008 with the publication of three families.7 Data assessing the prevalence of pathogenic rare variants in minor BrS genes indicate a total yield of less than 5%; the estimated disease prevalence of BrS5 is therefore at most 1% (1:200,000 [0.0005%]).4,7 There is alternate splicing of the *SCN1B* gene with an additional exon 3A in the alternate ß-1B transcript due to the retention of intron 3. Of note, one of the variants (Trp179X) from the seminal paper was only located on the alternatively spliced ß-1B transcript.8

BrS may be diagnosed in a family following an unexplained sudden cardiac death (Sudden Arrhythmic Death Syndrome [SADS]) and molecular autopsy.3,9 *SCN1B* is often included on routine genetic testing panels for BrS and SADS families with traditional Mendelian inheritance models. There is evidence that BrS is unlikely to be a monogenic disorder but rather an oligogenic disease with multiple rare and non-rare variants contributing to the underlying basis of disease.4,10 With ever increasing population-based datasets, some *SCN1B* variants previously implicated as monogenic causes of disease in BrS have been identified in high frequency in large population databases (http://gnomad.broadinstitute.org/). Additionally, the population datasets show higher than anticipated frequency of loss of function variants in *SCN1B*, suggesting loss of function may in fact be tolerated in this protein, particularly in the alternatively spliced ß-1B transcript.

Therefore, the aim of this study was to characterize genotype-phenotype correlation in BrS and SADS families with reported pathogenic *SCN1B* variants and to review their pathogenicity.

**METHODS**

**Patient selection and baseline assessment**

BrS and SADS families with potential pathogenic *SCN1B* variants were identified from six inherited arrhythmia centers worldwide. All affected and unaffected family members with genotyping data were included. Patients underwent baseline review with comprehensive history, physical examination and family pedigree. Baseline electrocardiogram (ECG) was reviewed and heart rate (HR), PR interval, QRS duration and corrected QT (QTc) intervals were recorded, as was the presence of a spontaneous type 1 Brugada pattern at baseline.1 ECGs with precordial leads placed in standard position and between 2nd and 4th intercostal spaces were reviewed. Any family members with no baseline spontaneous type 1 BrS pattern who had proceeded to drug provocation testing with type 1 anti-arrhythmic medications (either ajmaline 1mg/kg over 5 minutes or pilsicainide 1mg/kg 5 minutes) was noted and the results recorded. Any other unexpected findings on the baseline ECG were noted, specifically the presence of fragmented-QRS (defined as ≥4 spikes in one lead or ≥8 spikes in all leads V1-V3).11 Studies were approved by the Local Ethics Review Committee at St George’s University of London, United Kingdom.

**Genotype Evaluation in Families**

All families were initially screened for variants in *SCN5A*. Following negative *SCN5A* testing families proceeded to to further genetic testing with a minimum of 2 genes (*SCN5A*, *SCN1B*). Relevant genotyping results in *SCN1B* were recorded. The genotype-phenotype segregation of *SCN1B* variants within the family was reviewed.

**Pathogenicity Evaluation of *SCN1B* variants**

Normal population data for *SCN1B* variants were initially reviewed using the Genome Aggregation Database (gnomAD [http://gnomad.broadinstitute.org/about]).12 The general population minor allele frequency (and ethnicity specific minor allele frequency) for the potentially pathogenic *SCN1B* variants was recorded. The *SCN1B* transcript (ß-1 or ß-1B) and topological location of the variant within the protein was reviewed. The overall burden of loss of function variants in *SCN1B* was also evaluation using gnomAD browser. The *in silico* prediction for each variant was assessed using CADD (Combined Annotation-Dependent Depletion). Any previous literature on the variants was reviewed from ClinVar ([www.ncbi.nlm.nih.gov/clinvar/)](http://www.ncbi.nlm.nih.gov/clinvar/)) and any functional data in the literature were also noted. The variants were then reclassified using American College of Medical Genetics (ACMG) criteria by a single center.13

**Maximum credible population allele frequency for SCN1B variants**

The maximum tolerated allele frequency threshold for SCN1B variants in the general population was computed using a recently validated online tool (<http://cardiodb.org/alleleFrequencyApp)>.14 For the calculation the BrS5 disease prevalence was set at 1:200000, maximum penetrance=0.5, allelic heterogeneity 0.01 (indicating no single variant causes more than 1% of cases) and reference population size from gnomAD allele count.12,14

**Statistical Analysis**

Statistical analyses were carried out using SPSS (Version 23) and GraphPad Prism 7. Categorical variables were compared using Fisher’s exact tests. Significance was set at a two-sided p-value of <0.05.

**RESULTS**

**Patient selection and baseline assessment**

A total of 23 *SCN1B* genotype positive individuals were identified from 8 families worldwide. There was one family from Australia, the United Kingdom, Turkey, France, The Netherlands, Denmark, and two Japanese families. The largest family was from Australia with seven genotype positive family members. Baseline characteristics are shown in **Table 1**. The majority of the genotype positive individuals were male (13/23, 57%) with a mean age of 42±20 years. The majority (16/23, 70%) of the cohort were asymptomatic. Four (17%) probands experienced ventricular fibrillation at the time of presentation: one died suddenly (SADS) and three were successfully resuscitated. Of these four patients, one demonstrated a type 1 Brugada ECG pattern. Overall 5/23 (22%) genotype positive individuals demonstrated a type 1 Brugada ECG pattern, most of which (3/5, 60%) were spontaneous. The Turkish family had a phenotype consistent with progressive cardiac conduction disease (PCCD) rather than BrS. Of the family members who underwent drug challenge testing following positive predictive genetic testing results, 13/15 (87%) were negative i.e. a sensitivity of 13% for presence of a *SCN1B* variants.

**Genotype-Phenotype Evaluation in Families**

The pedigree of the three largest families are shown in **Figure 1A-C**, those with known phenotype and genotypes used in the study are highlighted. No BrS or SADS families showed any consistent segregation of genotype with phenotype. The Turkish kindred was known to have a progressive cardiac conduction disease (PCCD) phenotype and showed evidence of segregation. There was a total of 23 genotype positive and 3 genotype negative family members. The electrocardiographic phenotype was compared between genotype positive and genotype negative family members. There was no difference in PR (161±7 vs 165±9ms; p=0.83), QRS (101±6 vs 89±5ms; p=0.35) or QTc (414±35 vs 405±8ms; p=0.7) intervals between genotype positive and genotype negative family members.

**Evaluation of *SCN1B* variants**

The *SCN1B* variants seen among the 8 families are highlighted in **Table 2** and **Figure 2**. The most common *SCN1B* potential pathogenic variant, p.Trp179X, was present in 15/23 (65%) individuals from the Australian, British, Dutch and French families of Caucasian descent. The p.Trp179X variant is located within the retained intron 3 (exon 3A) in the alternatively spliced *SCN1B* transcript ß-1B. This variant is seen in gnomAD browser with an ethnicity specific minor allele count (MAC) of 2 and minor allele frequency (MAF) of 0.000018 amongst European non-Finnish population groups.12 The CADD score for this variant is 36 suggesting likely damaging effects on the protein. Previous functional data on the p.Trp179X variant have suggested co-expression of this mutant may lead to simple haploinsufficiency with mutant protein unable to modulate sodium channel function unlike wild type protein.7 The variant is a loss of function (nonsense) variant located in exon 3A. There are a total of 15 loss of function (frameshift, termination or splice-site) variants in *SCN1B* in the gnomAD browser with a total MAC of 33. The majority of these loss of function variants are found in the alternative 3A exon (11/15 (73%) variants; 28/33 (84%) count) suggesting that loss of function in this region of the gene is likely to be well tolerated (**Figure 2**).

Two variants were seen in the extracellular domain of the protein, p.Glu87Gln and p.Val158Met. Previous functional data exist on p.Glu87Gln variant, which demonstrated dominant negative effects of the mutant allele on the wild type allele with changes in voltage dependent inactivation of sodium channel gating.7 p.Glu87Gln is seen in gnomAD with a MAF 0.0012. p.Val158Met is absent from all control groups however an alternate nucleotide substitution leading to p.Val158Leu has a MAF 0.00004 in gnomAD browser. There were also two variants identified in the cytoplasmic domain, p.Thr189Met and p.Ala197Val with ethnicity specific MAF of 0.00019 and 0.000016 in gnomAD respectively for the ethnicities of the corresponding families in the study cohort. Finally, p.Arg214Gln also only found in the B1B transcript is seen frequently in gnomAD (MAF0.002) and has four submissions in ClinVar assessed as benign (SCV000291890.2, SCV000540265.1) or likely benign (SCV000342754.2, SCV000511289.1). The details of these variants are also shown in **Table 2**.

**Reclassification of Pathogenicity using ACMG criteria**

When reclassifying pathogenicity of the variants using ACMG criteria, none of the variants were graded higher than variants of uncertain significance [VUS] (**Table 2**). The p.Trp179X variant was not given ACMG very strong criteria for a null variant where loss of function is an established mechanism of disease (PVS1) as there is evidence from population databases that loss of function is well tolerated in the gene, particularly in the alternate 3A exon.11 The frequency of the variant exceeds what would be expected in the general population, based on the prevalence of Brugada syndrome (PM2). *In silico* data are supportive of a pathogenic role (PP3). Therefore, due to conflicting data the variant was downgraded to a VUS.

The p.Val158Met variant is absent from all general population data (PM2). Similarly p.Ala197Val is also absent in ethnically matched population data (PM2); however, lack of segregation or functional data mean this variant cannot be classified beyond VUS. The p.Arg214Gln variant has high frequency in population databases (BS1) and a number of benign literature reports (BP6) and is therefore reclassified as likely benign. The remaining variants are classified as likely benign due to higher than accepted frequencies for disease prevalence and *in silico* tools not suggesting pathogenic effects (BS1, BP4; **Table 2**).

**Maximum credible population AF for SCN1B variants**

The overall general population MAC of the *SCN1B* variants identified in our cohort is 515, giving an approximate global MAF of 0.001-0.002 (i.e. 1-2/1000).11 Furthermore, the most common variant identified amongst the families reviewed was p.Trp179X. This variant is seen in gnomAD with MAF 0.00002 (or 1/50,000). The computed maximum credible population allele frequency based on disease prevalence is 5x10-8 giving maximum tolerated allele count of zero.13

**DISCUSSION**

This study of *SCN1B* variants in BrS and SADS has highlighted the lack of evidence for these variants being monogenic causes for disease. Previously implicated *SCN1B* variants are found in unexpectedly high frequencies in general population databases, specifically gnomAD. Reclassification using ACMG criteria therefore led to downgrading of variants to VUS and likely benign.13 There is also poor evidence of genotype-phenotype correlation within the 8 families reviewed worldwide, including the three families from the original manuscript associating *SCN1B* with BrS and conduction disease. The UK family included a young autopsy negative sudden death victim who did not carry the *SCN1B* variant (genotype negative, phenotype positive, **Figure 1B**). Indeed, only the family with a PCCD phenotype showed any genotype-phenotype correlation amongst the families studied.8 Furthermore, a 13% sensitivity of drug challenge for presence of a *SCN1B* variant is inconsistent with incomplete penetrance when the sensitivity for *SCN5A* variants has been shown to be 80% using standard ECG lead placement.15 Previous functional data do show clear effects on sodium channel function of some implicated *SCN1B* variants, however, given their frequency it is more likely these variants are disease-modifiers rather than monogenic causes of disease.

**General population data**

Population datasets have recently evolved with the most recent release of gnomAD in October 2017 including a total dataset of 138,632 exomes and genomes from unrelated individuals.12 Within this dataset the overall MAC of the *SCN1B* variants previously implicated in disease is 515, giving an approximate MAF of 0.001-0.002 (i.e. 1-2/1000). This is more frequent than the accepted prevalence of BrS (0.0005, 1/2000) and is implausible as a monogenic cause of BrS.4,10 Furthermore, the most common variant identified amongst the families reviewed was p.Trp179X. This variant is seen in gnomAD with MAF 0.00002 (or 1/50,000), above the maximum tolerated allele count of zero using a validated computational threshold allele frequency model.14 Given the population frequency, this variant would therefore account for 1/25 (4%) cases of BrS which is also implausible.

p.Trp179X is found on an alternatively spliced *SCN1B* transcript ß-1B.8 The alternate transcript is present in the heart only in low levels, being most abundant in brain and skeletal muscle.16 Functional data from Watanabe et al, show that both *SCN1B* transcripts are expressed in cardiac tissue, with higher expression in Purkinje fibers than in cardiomyocytes, consistent with a conduction disease phenotype.8 In our cohort there was no segregation of any BrS phenotype, however the family with PCCD did show segregation, consistent with these findings. Furthermore, there was a trend towards QRS duration being longer in subjects with a *SCN1B* variant, although the small number of genotypically negative individuals limited the comparison.

Previous functional data suggest that haploinsufficiency is a mechanism of disease for the p.Trp179X variant. This variant is found within the alternate exon 3A, a region with a high burden of loss of function variants in control population data. In fact there are a total of 28 loss of function *SCN1B* variants in gnomAD, the majority found in the alternate 3A exon (11/15 [73%] variants; 28/33 [84%] count) suggesting that loss of function in this region of the gene may be well tolerated.12 The most common loss of function *SCN1B* variant seen in gnomAD, p.Arg261X, has a minor allele frequency of 0.0004% or 1/2500, further supporting this observation.12 A *SCN1B* knockout murine model has however highlighted the functional importance of the presence of some *SCN1B* for appropriate NaV1.5 channel function, at least in the mouse.17 The complete loss of *SCN1B* caused changes in sodium current, triggered beats, delayed after-depolarizations and increased susceptibility to ventricular arrhythmias, whilst Hu et al, demonstrated some reduction in sodium channel density with the R214Q polymorphism.18

Collectively these data suggest that the presence of *SCN1B* is important for sodium channel function, and that some of the variants previously implicated in BrS and SADS families are most likely disease modifiers of conduction rather than monogenic causes of BrS.

**Clinical implications**

There are important clinical implications of this study in individuals and families with BrS or SADS who have previously undergone predictive genetic testing. First, it is highly unlikely *SCN1B* is a monogenic cause of disease in these cases, therefore mutation negative individuals within a family should not be excluded from ongoing clinical screening. Family members who have previously been discharged from clinical screening may need to be re-reviewed for evidence of clinical disease. Our recommendation is that *SCN1B* no longer be considered causative for BrS when performing gene panel testing in BrS or SADS families.

**CONCLUSIONS**

The lack of genotype-phenotype concordance amongst families, combined with the high number of loss of function variants in the gene and frequency of previously reported mutations in the gnomAD browser suggests that *SCN1B* is not a monogenic cause for BrS or SADS cases. Therefore, we recommend against clinical diagnostic genetic testing for the *SCN1B* gene in these conditions.

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**REFERENCES**:

**1.** Antzelevitch C, Yan GX, Ackerman MJ, et al. J-Wave syndromes expert consensus conference report: Emerging concepts and gaps in knowledge. Heart Rhythm2016; 13(10):e295-324

**2.** 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 Cardiol1992;20:1391-1396.

**3.** Priori SG, Wilde AA, Horie M, et al. HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes. Heart Rhythm2013;10:1932-1963.

**4.** Gray B, Behr ER. New Insights Into the Genetic Basis of Inherited Arrhythmia Syndromes. Circ Cardiovasc Genet2016;9:569-577.

**5.** Wilde AA, Behr ER. Genetic testing for inherited cardiac disease. Nat Rev Cardiol 2013;10:571-583.

**6.** Probst V, Wilde AA, Barc J, et al. SCN5A mutations and the role of genetic background in the pathophysiology of Brugada syndrome. Circ Cardiovasc Genet2009;2:552-557.

7. Crotti L, Marcou AC, Tester DJ, Castelletti S, Giudicessi JR, Torchio M, Medeiros-Domingo A, Simone S, Will ML, Dagradi F, Schwartz PJ, Ackerman MJ. Spectrum and prevalence of mutations involving BrS1 through BrS12-susceptibility genes in a cohort of unrelated patients referred for Brugada syndrome genetic testing: implicatoins for genetic testing. J Am Coll Cardiol 2012:60:1410-8

**8.** Watanabe H, Koopmann TT, Le Scouarnec S, et al. Sodium channel beta1 subunit mutations associated with Brugada syndrome and cardiac conduction disease in humans. J Clin Invest2008;118:2260-2268.

**9.** Lahrouchi N, Raju H, Lodder EM, et al. Utility of Post-Mortem Genetic Testing in Cases of Sudden Arrhythmic Death Syndrome. J Am Coll Cardiol 2017;69:2134-2145.

**10.** Bezzina CR, Barc J, Mizusawa Y, et al. Common variants at SCN5A-SCN10A and HEY2 are associated with Brugada syndrome, a rare disease with high risk of sudden cardiac death. Nat Genet2013;45:1044-1049.

**11.** Morita H, Kusano KF, Miura D, Nagase S, Nakamura K, Morita ST, Ohe T, Zipes DP, Wu J. Fragmented QRS as a marker of conduction abnormality and a predictor of prognosis of Brugada syndrome. Circulation 2008;118:1697-1704.

**12.** Lek M, Karczewski KJ, Minikel EV, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature 2016;536:285-291.

**13.** Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med2015;17:405-424.

**14.** Whiffin N, Minikel E, Walsh R, O'Donnell-Luria AH, Karczewski K, Ing AY, Barton PJR, Funke B, Cook SA, MacArthur D, Ware JS. Using high-resolution variant frequencies to empower clinical genome interpretation. Genet Med 2017;19:1151-1158.

15. Hong K, Brugada J, Oliva A, Berruezo-Sanchez A, Potenza D, Pollevick GD, Guerchicoff A, Matsuo K, Burashnikov E, Dumaine R, Towbin JA, Nesterenko V et al. Value of electrocardiographic parameters and ajmaline test in the diagnosis of Brugada syndrome caused by SCN5A mutations. Circulation. 2004;110:3023-7

**16.** UniprotKB- Q07699 (SCN1B\_HUMAN). [http://www.uniprot.org/](http://www.uniprot.org). Accessed 13/12/17, 2017.

**17.** Lin X, O'Malley H, Chen C, Auerbach D, Foster M, Shekhar A, Zhang M, Coetzee W, Jalife J, Fishman GI, Isom L, Delmar M. Scn1b deletion leads to increased tetrodotoxin-sensitive sodium current, altered intracellular calcium homeostasis and arrhythmias in murine hearts. J Physiol 2015;593:1389-1407.

18. Hu D, Barajas-Martinez H, Medeiros-Domingo A, Crotti L, Veltmann C, Schimpf R, Urrutia J, Alday A, Casis O, Pfeiffer R, Burashnikov E, Caceres G, et al. A novel rare variant in SCN1Bb linked to Brugada Syndrome and SIDS by combined modulation of Nav1.5 and Kv4.3 channel currents. Heart Rhythm 2012;9:760-769

**FIGURE LEGEND**

**Figure 1:**

**(A) Family AUS pedigree (p.Trp179X); (B) Family UK pedigree (p.Trp179X); (C) Family Turkey pedigree (p.Glu87Gln)**

Squares = males; circles = females; line through a symbol = deceased individual; solid black symbols = clinically affected individuals; open symbols = clinically unaffected individuals; solid grey symbol = clinically borderline individual; arrow = proband; double line= consanguinity several generations back. +/- = presence of *SCN1B* variant; -/- = no variant identified.

**Figure 2:**

Overall SCN1B gene structure showing ß1 (top) and ß1B (below) transcripts. Exon 3A in ß1B transcript is due to alternate splicing and retention of intron 4. The variants seen in the study cohort are labelled. The red dots represent loss of function variants and the black dots below represent missense variants. Other loss of function variants seen in gnomAD for each transcript are represented by additional red dots. The key functional domains are labelled. TM- transmembrane; IC- intracellular.

**TABLE 1: Cases and Families for Putative Pathogenic *SCN1B* variants**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Family code** | **Age** | **Sex** | **Ethnicity** | ***SCN1B***  **Genotype** | **Type 1 Brugada pattern** | **Spontaneous/**  **Drug** | **Type 1 provocation test ?** | **Symptoms** | **HR** | **PR** | **QRS** | **QTc** | **Other** |
| AUS I-1 | 77 | M | European non-Finnish | -/- | no | n/a | No | No | 74 | 150 | 98 | 400 |  |
| AUS I-2 | 76 | M | European non-Finnish | Trp179X | No | n/a | no | no | 62 | 138 | 96 | 390 |  |
| AUS II-2 | 38 | M | European non-Finnish | Trp179X | No | n/a | yes | no | 54 | 152 | 98 | 380 |  |
| AUS II-3 | 49 | M | European non-Finnish | Trp179X | No | n/a | yes | no | 60 | 181 | 133 | 380 |  |
| AUS II-5 | 48 | F | European non-Finnish | Trp179X | Yes | drug | yes | no | 58 | 124 | 120 | 390 |  |
| AUS II-7\* | 42 | M | European non-Finnish | Trp179X | Yes | spontaneous | n/a | yes- RCA | 92 | 163 | 126 | 480 |  |
| AUS III-7 | 16 | M | European non-Finnish | Trp179X | No | n/a | yes | no | 48 | 170 | 110 | 393 |  |
| UK I-1 | 51 | M | European non-Finnish | -/- | No | n/a | yes | No | 78 | 166 | 90 | 416 |  |
| UK I-2 | 50 | F | European non-Finnish | Trp179X | No | n/a | yes | no | 66 | 128 | 90 | 430 |  |
| UK II-1 | 18 | M | European non-Finnish | Trp179X | No | n/a | yes | no | 66 | 110 | 100 | 420 |  |
| UK II-2\* | 21 | F | European non-Finnish | Trp179X | n/a | n/a | no | yes-SCD | n/a | n/a | n/a | n/a |  |
| Turk I-1 | 85 | F | Turkish | Glu87Gln | No | n/a | yes | no | 75 | 180 | 80 | 402 | Clinical AVNRT |
| Turk II-2\* | 61 | F | Turkish | Glu87Gln | No | n/a | yes | palpitation | 75 | 200 | 120 | 447 | LBBB and clinical AVNRT |
| Turk II-3 | 54 | M | Turkish | -/- | No | n/a | No | No | 75 | 180 | 80 | 400 |  |
| Turk II-4 | 54 | M | Turkish | Glu87Gln | Yes | drug | yes | no | 91 | 160 | 128 | 443 | Incomplete RBBB with LAD |
| Japan A1\* | 13 | F | East Asian | Thr189Met | No | n/a |  | yes- RCA | 83 | 160 | 105 | 459 |  |
| Japan B1\* | 34 | F | East Asian | Ala197Val | No | n/a |  | yes- RCA | 54 | 120 | 90 | 389 |  |
| Japan C1014 | 11 | M | East Asian | Val158Met | No | n/a | yes | no | 60 | 140 | 70 | 400 | Segregation of variant is on non-affected side of family |
| Japan C1015 | 29 | F | East Asian | Val158Met | No | n/a |  | no | 76 | 160 | 60 | 400 |  |
| Denmark 3\* | 54 | M | European non-Finnish | Arg214Gln | Yes | spontaneous | n/a | presyncope |  | 200 |  |  |  |
| France II-4\* | 52 | M | European non-Finnish | Trp179X | Yes | spontaneous | n/a | chest pain | 60 | 220 | 120 | 400 |  |
| France II-1 | 53 | M | European non-Finnish | Trp179X | No | n/a | yes | no | 60 | 180 | 80 | 380 | LAD, type 2 BrS pattern baseline and with flecainide, no type 1 |
| France II-2 | 58 | F | European non-Finnish | Trp179X | No | n/a | yes | no | 62 | 146 | 96 | 440 |  |
| France III-1 |  | M | European non-Finnish | Trp179X | No | n/a | Yes | no |  |  |  |  | RBBB |
| Dutch II-1\* | 17 | F | European non-Finnish | Trp179X | No | n/a | yes | no | 74 | 196 | 116 | 492 | 1st degree AVB, RBBB |
| Dutch I-1 |  | M | European non-Finnish | Trp179X | No | n/a | yes | no | 90 | 160 | 80 | 360 |  |

Table Legend: \*indicates proband, HR- heart rate, PR- PR interval (ms), QRS- QRS duration (ms), QTc- Corrected QT interval (ms), RCA- resuscitated cardiac arrest, SCD- sudden cardiac death, AVNRT- atrioventricular nodal reentry tachycardia, L/RBBB- left/right bundle branch block, LAD- left axis deviation, AVB- atrioventricular block

**TABLE 2: Evaluation/Pathogenicity assessment of *SCN1B* variants**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Variant**  **(GRCh37/hg19)** | **Variant**  **p. AA change** | **dbSNP** | **Overall**  **gnomAD MAC** | **Overall**  **gnomAD MAF**  **(total alleles)** | **Ethnicity specific**  **gnomAD MAF (n=total alleles)** | **CADD** | **Topology** | **Other** | **ACMG criteria** | **ACMG**  **classification** |
| 19:35524454G>A | Glu87Gln | rs121434627 | 3 | 0.0012 | 0.0000  (no Turkish specific data) | 15 | Extracellular immunoglobulin loop | Functional data show effect on Nav1.5 gating7 | BS1  BP4 | Likely Benign |
| 19:35530044G>A | Val158Met | rs138450474 | 0 | 0 | 0  (n=17248) | 18 | Extracellular | p.Val158Leu gnomAD MAF 0.00004 | PM2 | VUS |
| 19:35524731G>A | Trp179X | rs267607028 | 2 | 0.000008 | 0.000018  (n=110752) | 36 | Exon 3A  ß-1B transcript | Functional data show effect on  Nav1.5 current8 | PM2  PP3 | VUS |
| 19:35530138C>T | Thr189Met | rs2305748 | 53 | 0.00019 | 0.0019  (n=18870) | 16 | Cytoplasmic | gnomAD ethnicity MAC 37 | BS1  BP4 | Likely Benign |
| 19:35530162C>T | Ala197Val | rs554201948 | 4 | 0.000016 | 0  (n=17248) | 16 | Cytoplasmic |  | PM2 | VUS |
| 19:35524836G>A | Arg214Gln | rs66876876 | 453 | 0.002 | 0.004  (n=77874) | 11 | Exon 3A  ß-1B transcript | Functional data show reduction in Nav 1.5 density and 71% increase in Kv 4.318 | BS1  BP6 | Likely Benign |