American Heart Association.

Circulation: Genomic and Precision Medicine

Manuscript Submission and Peer Review System

URL: https://circgenetics-submit.aha-journals.org

Title: Genotype-Phenotype Correlation of SCN5A Genotype in Patients with Brugada Syndrome and Arrhythmic Events: Insights from the Survey on Arrhythmic Events in Brugada Syndrome (SABRUS) in 392 Probands.

Manuscript number: CIRCCVG/2020/003222R3

Author(s): Bernard Belhassen, Hadassah University Hospital

Anat Milman, Leviev Heart Center , Sheba Medical Center and Sackler Faculty of Medicine, Tel Aviv University

Elijah Behr, St George's University of London

Belinda Gray, St George's, University of London

David Johnson, Cardiovascular Sciences and Cardiology Clinical Academic Group St. George's University Hospitals NHS Foundation Trust, London, UK.

Antoine Andorin, Institut du Thorax - CHU Nantes
Aviram Hochstadt, , Tel-Aviv Sourasky Medical Center and the Sackler school of medicine of The Tel Aviv university
Jean-Baptiste Gourraud, l'institut du thorax, INSERM, CNRS, UNIV Nantes, CHU Nantes, Nantes, France
Shingo Maeda, Tokyo Medical and Dental University
Yoshihide Takahashi, Tokyo Medical and Dental University
Jyh-Ming Juang, Cardiovascular Center and Division of Cardiology, Department of Internal Medicine, National Taiwan University Hospital and National Taiwan University College of Medicine
Sung-Hwan Kim, Seoul St.Mary's Hospital, Catholic University
Shiro Kamakura, National Cerebral and Cardiovascular Center
Takeshi Aiba, National Cerebral and Cardiovascular Center
Pieter Postema, Amsterdam University Medical Centers
Yuka Mizusawa, Academic Medical Center
Isabelle Denjoy, cService de Cardiologie et CNMR Maladies Cardiaques Héréditaires Rares, Hôpital Bichat
Carla Giustetto, University of Torino
Giulio Conte, Istituto Cardiocentro Ticino, Ente Ospedaliero Cantonale
Zhengrong Huang, the First Affiliated Hospital of Xiamen University
Georgia Sarquella-Brugada, Hospital Sant Joan de Deu
Andrea Mazzanti, IRCCS ICS Maugeri
Camilla Jespersen, The Department of Cardiology, The Heart Centre, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark
Elena Arbelo, Cardiovascular Institute - Hospital Clinic, Universitat de Barcelona

Ramon Brugada, Cardiology Department, Hospital Trueta, Department of Medical Sciences, University of Girona, and IDIBGI, Girona

Leonardo Calò, Policlinico Casilino

Domenico Corrado, University of Padova

Ruben Casado Arroyo, Université Libre de Bruxelles

Giuseppe Allocca, xDivision of Cardiology, General Hospital of Conegliano (Treviso), Italy

Masahiko Takagi, Kansai Medical University

Pietro Delise, Hospital Pederzoli

Josep Brugada, Cardiovascular Institute, Hospital Clinic and IDIBAPS, Barcelona, Catalonia

Jacob Tfelt-Hansen, Copenhagen University Hospital, Rigshospitalet

Silvia Priori, IRCCS Istituti Clinici Scientifici Maugeri University of Pavia

Christian Veltmann, Hannover Medical School

Gan-Xin Yan, Lankenau Medical Center

Pedro Brugada, Heart Rhythm Management Centre

Fiorenzo Gaita, University of Turin, Turin, Italy

Antoine Leenhardt, Hôpital Bichat, APHP

Arthur Wilde, Academic Medical Center, Amsterdam

Kengo Kusano, Division of Arrhythmia and Electrophysiology, Department of Cardiovascular Medicine, National Cerebral and Cardiovascular Center

Gi-Byoung Nam, University of Ulsan College of Medicine

Kenzo Hirao, Tokyo Medical & Dental University

Vincent Probst, Service de Cardiologie, CHU de Nantes, Nantes, France

FINAL MANUSCRIPT (after acceptance June 21, 2021)

Genotype-Phenotype Correlation of *SCN5A* Genotype in Patients with Brugada Syndrome and Arrhythmic Events: Insights from the Survey on Arrhythmic Events in Brugada Syndrome (SABRUS) in 392 Probands.

Milman. Genotype-Phenotype Correlation in Brugada Syndrome

Anat Milman MD PhD[1,2], Elijah R. Behr MD[3,4], Belinda Gray MD PhD[4], David C Johnson[3,4], Antoine Andorin MD[3,5], Aviram Hochstadt MD[2,6], Jean-Baptiste Gourraud MD PhD[3,5], Shingo Maeda MD PhD[7], Yoshihide Takahashi MD PhD[7], Jimmy JM Juang MD PhD[8], Sung-Hwan Kim MD[9], Tsukasa Kamakura MD PhD[10], Takeshi Aiba MD PhD[10], Pieter G. Postema MD PhD[3,11], Yuka Mizusawa MD[3,11], Isabelle Denjoy MD[12], Carla Giustetto MD[13], Giulio Conte MD PhD[14], Zhengrong Huang MD PhD[15], Georgia Sarquella-Brugada MD PhD[16], Andrea Mazzanti MD[3,17], Camilla H. Jespersen MD[3,18,19], Elena Arbelo MD PhD[20], Ramon Brugada MD PhD[21,22,23], , Leonardo Calo MD[24], Domenico Corrado MD PhD[3,25], Ruben Casado-Arroyo MD PhD[26], Giuseppe Allocca MD[27], Masahiko Takagi MD PhD[28], Pietro Delise MD[27], Josep Brugada MD PhD[20] , Jacob Tfelt-Hansen MD DMSc[3,18,19], Silvia G. Priori MD PhD[3,17], Christian Veltmann MD[29], Gan-Xin Yan MD PhD[30], Pedro Brugada MD PhD[14], Fiorenzo Gaita MD[13], Antoine Leenhardt MD[12], Arthur A.M. Wilde MD PhD[3,11], Kengo F. Kusano MD PhD[10], Gi-Byoung Nam MD PhD[31], Kenzo Hirao MD PhD[7], Vincent Probst MD PhD[3,5], Bernard Belhassen MD[2,32]

[1] Leviev Heart Institute, The Chaim Sheba Medical Center, Tel Hashomer, Israel

[2] Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel

[3] European Reference Network for Rare and Low Prevalence Complex Diseases of the Heart

[4] Cardiovascular Clinical Academic Group, St George's, University of London and St. George's University Hospitals NHS Foundation Trust, London, UK

[5] Service de Cardiologie, CHU de Nantes, Nantes, France

[6] Department of Cardiology, Tel-Aviv Sourasky Medical Center, Tel Aviv, Israel

[7] Heart Rhythm Center, Tokyo Medical and Dental University, Tokyo, Japan

[8] Cardiovascular Center and Division of Cardiology, National Taiwan University Hospital and University College of Medicine, Taipei, Taiwan

[9] Division of Cardiology, College of Medicine, The Catholic University of Korea, Seoul, Korea

[10] Division of Arrhythmia and Electrophysiology, National Cerebral and Cardiovascular Center, Osaka, Japan

[11] Amsterdam UMC, University of Amsterdam, Heart Center; department of Clinical and Experimental Cardiology, Amsterdam, The Netherlands

[12] Service de Cardiologie et CNMR Maladies Cardiaques Héréditaires Rares, Hôpital Bichat, Paris, and Université Paris Diderot, Sorbonne, Paris, France

[13] Division of Cardiology, University of Torino, Department of Medical Sciences, Città della Salute e della Scienza Hospital, Torino, Italy

[14] Heart Rhythm Management Centre, UZ-VUB, Brussels, Belgium

[15] Department of Cardiology, the First Affiliated Hospital of Xiamen University, Xiamen, Fujian, China

[16] Pediatric Arrhythmias, Electrophysiology and Sudden Death Unit Cardiology, Department Hospital Sant Joan de Déu, Barcelona - Universitat de Barcelona, Spain

[17] Molecular Cardiology, Istituti Clinici Scientifici Maugeri IRCCS, Pavia, Italy

[18] The Heart Centre, Copenhagen University Hospital, Copenhagen, Denmark

[19] Department of Forensic Medicine, Faculty of Medical Sciences, University of Copenhagen, Copenhagen, Denmark

[20] Cardiovascular Institute, Hospital Clinic and IDIBAPS, Barcelona, Catalonia, Spain

[21] Cardiovascular Genetics Center, University of Girona-IDIBGI, Girona, Spain

[22] Medical Science Department, School of Medicine, University of Girona, Girona, Spain

[23] Centro de Investigación Biomédica en Red de Enfermedades Cardiovasculares (CIBERCV), Madrid, Spain

[24] Division of Cardiology, Policlinico Casilino, Roma, Italy

[25] Department of Cardiac, Thoracic and Vascular Sciences University of Padova, Padova, Italy

[26] Department of Cardiology, Erasme University Hospital, Université Libre de Bruxelles, Brussels, Belgium

[27] Division of Cardiology, Hospital of Peschiera del Garda, Veneto, Italy

[28] Division of Cardiac Arrhythmia, Kansai Medical University Medical Center, Moriguchi, Japan

[29] Hannover Heart Rhythm Center, Department of Cardiology and Angiology, Hannover Medical School, Hannover, Germany

[30] Lankenau Medical Center, Wynnewood, Pennsylvania, USA

[31] Division of Cardiology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

[32] Heart Institute, Hadassah University Hospital, Jerusalem, Israel.

Address for correspondence:

Bernard Belhassen, MD
Heart Institute,
Hadassah University Hospital
Jerusalem, Israel.
Telephone: +972-52-4-266-856, Fax: +972-153-52-4266856
Email: bblhass@gmail.com; bblhass@hadassah.org.il

Total word count – 5089

Journal Subject Terms: Genetics; Sudden cardiac death

Abstract

Background:

Brugada syndrome (BrS) is associated with mutations in the cardiac sodium channel gene, *SCN5A.* However, genetic studies of BrS patients with arrhythmic events (AE) have been limited. We sought to compare various clinical, ECG and EP parameters according to *SCN5A* genotype in a large cohort of BrS probands with first AE.

Methods:

SABRUS is a survey of 10 Western and 4 Asian countries, gathering 678 BrS patients with first AE. Only probands were included and *SCN5A* genotype adjudicated. Patients without appropriate genetic data were excluded. Associations of genotype with clinical features were analyzed.

Results:

The study group comprised 392 probands: 92 (23.5%) *SCN5A+* [44 pathogenic/likely pathogenic (P/LP) and 48 variants of unknown significance] and 300 (76.5%) *SCN5A-. SCN5A* missense variants and the patients hosting them were similar regardless of adjudication. A higher proportion of P/LP patients were pediatric (<16 years) compared to *SCN5A-* (11.4% vs. 3%, p=0.023). The proportion of females was higher amongst P/LP patients compared to *SCN5A-* (18.2% vs. 6.3%, p=0.013). *P/LP* probands were more likely to have a family history of sudden cardiac death (FHSCD) compared to *SCN5A-* (41.9% vs. 16.8%, p<0.001). A higher proportion of *P/LP* patients were Caucasian compared to *SCN5A-* (87.5% vs. 47%, p<0.001). Ethnicity (OR=5.41 [2.8-11.19], p<0.001) and FHSCD (OR of 2.73 [1.28-5.82], p=0.009) were independent variables associated with *P/LP* genotype following logistic regression.

Conclusions:

The genetic basis of BrS has a complex relationship with gender, ethnicity, and age. Probands hosting a P/LP variant tended to experience their first AE at a younger age and to have events

triggered by fever compared to *SCN5A*- patients. In addition, they were more likely to be Caucasian and to have FHSCD. Amongst females, a P/LP variant suggests an increased risk of being symptomatic. This association should be further studied on an ethnically specific basis in large prospectively collected international cohorts.

Key Words: Brugada syndrome; Arrhythmia.

Non-standard Abbreviations and Acronyms

ACA = aborted cardiac arrest

AE = arrhythmic event

BrS = Brugada syndrome

ECG = electrocardiographic

EP = electrophysiologic

EPS = electrophysiologic study

GWAS = genome wide association study

ICD = implanted cardioverter-defibrillator

LP = likely pathogenic

P = pathogenic

SCD = sudden cardiac death

VF = ventricular fibrillation

VUS = variant of uncertain significance

Introduction

Brugada syndrome (BrS) is an inherited channelopathy associated with increased risk of malignant ventricular tachyarrhythmias and sudden cardiac death (SCD)[1]. The condition typically affects apparently healthy young males in their 40's who exhibit a typical spontaneous or drug-induced ST elevation in at least one of the right precordial ECG leads (V1 through V3). The clinical phenotype and its manifestations have been reviewed in detail elsewhere[2].

The *SCN5A* gene was the first associated with the disease[3] and encodes the alpha-subunit of the cardiac sodium channel Nav1.5, responsible for the sodium inward current (INa). Implicated rare pathogenic variants (mutations) in *SCN5A* exhibited a reduction of INa, and consequently led to slowing of conduction. Rare variants in more than 20 genes encoding other channels or channel interacting proteins, have also been linked to BrS[4]. Disease-gene associations were, however, recently re-evaluated by an expert consortium of the Clinical Genomic Resource. This panel concluded that only the *SCN5A* gene has a rigorous enough level of genetic and experimental evidence to offer any confidence regarding disease causality[5]. The remaining heritability of BrS appears to be due to common genetic variation[6], suggesting a complex oligogenic architecture underlying genetic susceptibility to BrS[7].

Genotype-phenotype correlation of *SCN5A* mutation in BrS patients has been the subject of numerous studies. Chen and coworkers recently reported a meta-analysis[8] and found that *SCN5A* mutation carriers had a younger age at onset of symptoms, higher spontaneous type-1 ECG pattern and more pronounced conduction and repolarization abnormalities; in addition, the presence of *SCN5A* mutations was associated with an increased risk of major arrhythmic events (AEs) in both Asians and Caucasians. However, all studies included in this meta-analysis involved BrS patients with various clinical presentations, with only a small proportion of patients with documented AEs.

SABRUS[9-12] is the largest multicenter Survey on Arrhythmic Events in BRUgada Syndrome, including 678 BrS patients with documented AEs. The present study sought to assess the correlation of various clinical, ECG and EP parameters with *SCN5A* mutation status in a large cohort of patients with first AE. In order to avoid selection bias of including affected family members, only probands were studied.

Methods

The data that support the findings of this study are available from the corresponding author upon reasonable request. The authors declare that all supporting data are available within the article [and its online supplementary files]. The full methods are available as supplemental data. The study was approved by the Research Ethics Boards of all participating institutions.

Results

The study group comprised 392 SABRUS probands: 92 (23.5%) *SCN5A+* and 300 (76.5%) *SCN5A-*. The overall demographics of the entire cohort are presented in **Table 1**. The demographic, clinical, ECG, and EPS results of the study patients according to *SCN5A* genotype status are presented in **Table 2 and supplemental Table I.**

Age at time of AE.

Patients with P/LP variants were significantly younger than the *SCN5A*- group (36.6 $\pm$ 17.2 vs. 42.2 $\pm$ 14.0 years old, P=0.018) (**Table 2B).** Most patients in both groups were >16 years old, however, a higher proportion of pediatric patients (<16 years) were observed in the *P/LP* group compared to the *SCN5A*- group (11.4% vs. 3%, p=0.023). **Figure 1** displays *SCN5A* genotype according to age group at time of AE. The highest proportion of *SCN5A*+ patients was observed in the pediatric age group ($p<0.05$).

Gender and ethnicity

The proportion of females was significantly higher amongst the *P/LP* compared to the *SCN5A*- groups (18.2% vs. 6.3% respectively, p=0.013) (**Table 2**). Additionally, the proportion of *P/LP* in females (29.6%) was also significantly higher than that in males (11.36%) (P = 0.0063). Out of the 392 study group patients, 205 (52.3%) were Caucasian, 162 (41.3%) were of Asian ethnicity and the remainder 25 patients (6.4%) were of other or unknown ethnicity. When studying Asian and Caucasian ethnicity, a significantly higher proportion of *P/LP* patients were Caucasian compared to *SCN5A*- patients (87.5% vs. 47%, p<0.001); however, the opposite was observed for Asian patients (12.5 % vs. 53% p<0.001). Moreover, a pathogenic or likely pathogenic *SCN5A* variant was present in only 5 (3%) of all Asian patients compared to 31 (15.1%) of all Caucasian patients (p<0.001).

Family History

Although most study patients did not have a family history of SCD (77.3%), *P/LP* probands had a higher percentage of such a family history than *SCN5A*- probands (41.9% vs. 16.8%, respectively, p<0.001).

Other findings

No difference was observed in first AE presentation (group A and group B) (p=0.161). Furthermore, the proportion of *P/LP* patients who had their AE during fever was almost twice than that of *SCN5A*- patients (10.3% vs. 4.5%) but the difference was also not statistically significant (p=0.137).

No difference was observed between the groups with respect to a prior history of syncope before the AE (38.6% vs. 34%, respectively, p=0.611). A similar high prevalence of spontaneous type1 BrS-ECG was observed in *P/LP* and in *SCN5A*- patients (68.2% vs. 65.7%, respectively, p=0.865). EPS was performed in the same proportion of patients for both groups, with a comparable proportion of patients having inducible VF during the test (56.7% and 62.8%, respectively, p=0.545). No significant

difference in the clinical, ECG and EPS results were observed in respect of the type of *SCN5A* variant (**supplemental Table II**).

Pathogenic/likely pathogenic variants compared to VUS

Patients with a VUS (n=48) were similar in their clinical characteristics to patients who harbored P/LP variants (n=44) (**supplemental Table III**) except for a family history of SCD (18.2% vs 41.9% respectively, p=0.02). Furthermore, there was no difference in the transmembrane topological location of missense variants deemed P/LP compared to VUS (14/18 [78%] vs 31/41 [76%] p=1). An additional comparison of the VUS group to the SCN5A- group (**supplemental Table IV)** yielded a difference in ethnicity (p<0.001) with a trend towards a younger age (<16 years) for the VUS group (p=0.089).

Multivariate analysis

A logistic regression was performed for prediction of a P/LP *SCN5A* variant (P/LP vs. *SCN5A*-) (**Table 3**). The two independent factors observed were ethnicity, with Caucasians having an OR of 5.09 [1.97-15.8] (p=0.002) for a P/LP variant and family history of SCD with an OR of 2.73 [1.28-5.82] (p=0.009) for an LP/P variant. Caucasian ethnicity was also observed as an independent factor when comparing patients with VUS to the *SCN5A*- group (**supplemental Table V**).

Discussion

The present study, conducted in the largest BrS population with first AE ever reported, aimed to assess various clinical, ECG and EPS parameters according to *SCN5A* genotype. There are 3 main findings: 1) probands hosting a P/LP variant experience their first AE at a younger age than *SCN5A*-patients with a particular importance in the pediatric age group; 2) P/LP probands are characterized by higher proportions of females, Caucasians and a positive family history of SCD, the latter two factors being independent at multivariable analysis; 3) Probands hosting P/LP variants were similar

although not identical to those hosting a VUS, as was the topological location of missense P/LP and VUS variants.

This last finding reflects that a rare *SCN5A* variant in a definite case of BrS has a higher *a priori* likelihood of being disease associated than represent background population genomic variation[13,14]. Nonetheless, there are still benign variants amongst the VUS group that weaken some of the associations identified in the comparison of the P/LP group with the VUS group.

Previous studies.

Most previous prognostic studies did not find that *SCN5A* genotype influenced AE rate. These included large European surveys[15,16,17,18] and concluded that BrS patients who carry a pathogenic *SCN5A* variant have more pronounced cardiac conduction defects than BrS patients without. In addition, patients with loss of function alleles causing haploinsufficiency showed more severe conduction disorders but no increased risk of SCD[19]. Moreover, a meta-analysis of the main BrS databases available reported no difference in the risk of life-threatening arrhythmias in *SCN5A* genotype positive subjects[20].

In contrast, in a recent Japanese study of BrS probands, 97% of whom were male, Yamagata et al.[21] showed that the presence of a pathogenic *SCN5A* variant, using quite stringent criteria, was a significant predictor of arrhythmic events. Additionally, two recent meta-analyses obtained new insights when comparing BrS patients harboring an *SCN5A* variant to those without. The first performed by Yang et al.[22] intended to elucidate the effect of *SCN5A* genotype on BrS patients of mixed ethnicity with symptoms and/or undergoing EPS. They concluded that in symptomatic patients or those with a negative EPS, the presence of an *SCN5A* variant conferred a higher risk of arrhythmic events. Ethnicity was not examined. The second meta-analysis by Chen et al.[8] observed that positive *SCN5A* genotype was associated with an elevated risk of major AEs in both Caucasian and Japanese patients. None of these studies re-evaluated *SCN5A* variants according to ACMG criteria[23].

Present study.

The present study is unique and different from previous publications due to the large cohort of BrS probands with a documented AE. Our patient cohort therefore has pre-selected higher risk enabling several important observations. We were also able to re-evaluate *SCN5A* variants systematically for pathogenicity according to ACMG criteria.

Age at time of AE. Our study observed a younger age at time of AE in probands with a P/LP *SCN5A* variant. This result is in concordance with the meta-analysis by Chen et al.[8] where mutation carriers had a younger age at symptom presentation. We found that the younger age derived from a higher proportion of pediatric patients harboring a *SCN5A* variant. A similar observation was previously reported in a prospective study by Andorin et al.[24], where 9 of the 10 pediatric patients who had a life-threatening AE during follow up were found to carry the *SCN5A* mutation (90%). Our results as well as those of Andorin et al.'s[24] should encourage further prospective studies in larger groups of pediatric patients.

Gender. In a previous analysis[11] we observed that the proportion of male SABRUS-patients with *SCN5A* variants was slightly higher than that of a large cohort of asymptomatic male BrS-patients (27.8% vs. 20.8%, P<0.001). However, in female SABRUS-patients the proportion with *SCN5A* variants was markedly higher than in asymptomatic female BrS-patients (47.6% vs. 26.8%, P<0.001), suggesting, for the first time, that *SCN5A* genotype could represent an important risk marker for AE in females and to a lesser extent in male patients. In the whole patient group, an *SCN5A* mutation was found almost twice as much in females (48% vs 28% in males; P=0.007). In our present study involving probands only, a significantly higher proportion of P/LP *SCN5A* variant carriers was observed in the female cohort compared to males (more than twice as much), strengthening the hypothesis that *SCN5A* variants are an important risk marker for AE in females, as well as genetic susceptibility to BrS. These results highlight the important role that sex hormones play in BrS phenotype, resulting in opposite effects on the age of presentation and at onset of AEs.

Testosterone plays a crucial role in Brugada male phenotype influencing the age at onset of AE in adulthood[25-27]. Females, however, experience AEs at younger and older ages, in association with greater genetic susceptibility in the form of *SCN5A* variants. However, when estrogen levels are higher after puberty and before menopause, there may be a protective effect as observed by Song et al.[28].

Family history of SCD. For the first time, our study observed a significant association between P/LP *SCN5A* genotype in probands and a family history of SCD. This correlation is intuitive in that there may be a greater chance of genetic heritability of BrS and therefore risk of having BrS in families with *SCN5A* variants. Our data also therefore suggest that this results in a more severe form of the disease in family members, potentially due to a more penetrant genetic lesion. Our results contrast with those of Yamagata et al.[21], who did not observe such a difference. Moreover, the meta-analysis by Yang et al.[22] did not find an association between family history of SCD and future AE in subjects harboring *SCN5A* variants. Finally, the most recent meta-analysis by Chen et al.[8] also did not find a difference in the family history of SCD according to genotype. We speculate that these differences could be due to our population being already high-risk (i.e., all with AEs) and the use of ACMG criteria to determine pathogenicity.

Ethnicity. Ethnicity was an independent and consistent predictor for a *SCN5A* variant, whether P/LP or VUS. Caucasians had a significantly higher chance (OR 5.41) of harboring a P/LP variant. BrS is more prevalent in Asian countries[29], however the proportion of Asian patients with rare *SCN5A* variants is lower[12]. This was observed for the entire SABRUS cohort[12] as well as for probands in the present study. Chen et al.[8] described that *SCN5A* genotype was associated with an elevated risk of AEs in both Asian and Caucasian patients. In our study, the discrepancy in *SCN5A* variants between Asian and Caucasian patients, all with AEs, could theoretically be attributed to relative higher susceptibility of Asian patients to BrS due to environmental effects and a different genetic

architecture of oligogenic risk[30] that may already place them at a higher risk, rendering a rare *SCN5A* variant less important in Asian Brugada syndrome patients.

Supportive data for relevant differences in genetic architecture between Asian and Caucasian patients have been identified previously. A promoter region haplotype for the *SCN5A* gene was found to be common in the Japanese population yet absent in Caucasians and Blacks. This caused reduced expression of *SCN5A* and was associated with increased conduction abnormalities in general population and BrS patients[31]. More recently, Juang et al.[32] reported that common single nucleotide polymorphisms previously associated with BrS in the Caucasian and Japanese populations[6] were also overrepresented in Taiwanese BrS patients when compare to the Taiwanese general population in a genome wide association study (GWAS). However, their cumulative effect on disease risk was greatest in *SCN5A*- BrS patients. This was not noted in the previous GWAS[6], suggesting that there may be a stronger effect in the Taiwanese population. Furthermore, in the Thai population, rare *SCN5A* variants were only found in 6% of cases but low frequency and common genetic variation appeared to be significant contributors[33].

Our results support the observation that different ethnic groups have differing genetic architecture associated with risk in BrS. The clinical implication of such an observation, if validated in prospective studies, could imply that risk stratification in BrS should be studied separately for each ethnic group.

Study limitations

The present study has several limitations including its retrospective nature. Moreover, we did not test for the presence of several ECG characteristics known to correlate with *SCN5A* mutation carriers, such as conduction disturbances[19] and presence of late potentials on signal-averaged ECG[34].

Summary

The genetic basis of Brugada syndrome is oligogenic, in which *SCN5A* plays a critical role, and has a complex relationship with gender, ethnic origin and age. Furthermore, when a VUS is identified in

SCN5A in a severely affected individual, it is more likely to be disease associated than benign. Nonetheless, when evaluation with ACMG criteria indicates that a variant is pathogenic or likely pathogenic, the associations with ethnicity, age, gender, and family history of SCD are at their strongest. Probands hosting a P/LP variant tended to experience their first AE at a younger age and to have events triggered by fever compared to *SCN5A*- patients. In addition, they were more likely to be Caucasian and to have a positive family history of SCD. Amongst females, a P/LP variant suggests an increased risk of being symptomatic. This association should be further studied on an ethnically specific basis in large prospectively collected international cohorts.

Funding: None

Disclosures: All authors declare having no potential conflict of interest.

Supplemental Materials:

Supplemental Methods

Supplemental Tables I-VI

Supplemental Figures I-II

References

1. 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.

2. Brugada J, Campuzano O, Arbelo E, Sarquella-Brugada G, Brugada R. Present status of Brugada syndrome: JACC state-of-the-art review. *J Am Coll Cardiol* 2018;72:1046-1059.

3. Chen Q, Kirsch GE, Zhang D, Brugada R, Brugada J, Brugada P, Potenza D, Moya A, Borggrefe M, Breithardt G et al. Genetic basis and molecular mechanism for idiopathic ventricular fibrillation. *Nature* 1998;392:293-296.

4. Watanabe H, Minamino T. Genetics of Brugada syndrome. *J. Hum. Genet.* 2016;61:57-60.

5. Hosseini SM, Kim R, Udupa S, Costain G, Jobling R, Liston E, Jamal SM, Szybowska M, Morel CF, Bowdin S et al. Reappraisal of reported genes for sudden arrhythmic death. *Circulation* 2018;138:1195-1205.

6. Bezzina CR, Barc J, Mizusawa Y, Remme CA, Gourraud JB, Simonet F, Verkerk AO, Schwartz PJ, Crotti L, Dagradi F 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 Genet.* 2013;45:1044-1049.

7. Behr ER. Genetic susceptibility and the Brugada syndrome. *Eur Heart J.* 2019;40:3094-3096.

8. Chen C, Tan Z, Zhu W, Fu L, Kong Q, Xiong Q, Yu J, Hong K. Brugada syndrome with SCN5A mutations exhibits more pronounced electrophysiological defects and more severe prognosis: A meta-analysis. *Clin. Genet.* 2020;97:198-208.

9. Milman A, Andorin A, Gourraud JB, Sacher F, Mabo P, Kim SH, Maeda S, Takahashi Y, Kamakura T, Aiba T et al. Age of first arrhythmic event in Brugada syndrome: data from the SABRUS (Survey on Arrhythmic Events in Brugada Syndrome) in 678 patients. *Circ Arrhythm Electrophysiol* 2017;10:1-15.

10. Milman A, Andorin A, Gourraud JB, Postema PG, Sacher F, Mabo P, Kim SH, Juang JJM, Maeda S, Takahashi Y et al. Profile of patients with Brugada syndrome presenting with their first documented arrhythmic event: Data from the Survey on Arrhythmic Events in BRUgada Syndrome (SABRUS). *Heart Rhythm* 2018;15:716-724.

11. Milman A, Gourraud JB, Andorin A, Postema PG, Sacher F, Mabo P, Conte G, Giustetto C, Sarquella-Brugada G, Hochstadt A et al. Gender differences in patients with Brugada syndrome and arrhythmic events: Data from a survey on arrhythmic events in 678 patients. *Heart Rhythm* 2018;15:1457-1465.

12. Milman A, Andorin A, Postema PG, Gourraud JB, Sacher F, Mabo P, Kim SH, Maeda S, Takahashi Y, Kamakura T et al. Ethnic differences in patients with Brugada syndrome and arrhythmic events: New insights from Survey on Arrhythmic Events in Brugada Syndrome. *Heart Rhythm* 2019;16:1468-1474.

13. Kapplinger JD, Giudicessi JR, Ye D, Tester DJ, Callis TE, Valdivia CR, Makielski JC, Wilde AA, Ackerman MJ. Enhanced classification of Brugada syndrome-associated and Long-QT

syndrome-associated genetic variants in the SCN5A-encoded NA(v)1.5 cardiac sodium channel. *Circ Cardiovasc Genet*. 2015;8:582-595.

14. Ackerman MJ, Priori SG, Willems S, Berul C, Brugada R, Calkins H, Camm AJ, Ellinor PT, Gollob M, Hamilton R et al. HRS/EHRA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies: this document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA). *Europace*. 2011;13:1077-1109.

15. Priori SG, Gasparini M, Napolitano C, Della Bella P, Ottonelli AG, Sassone B, Giordano U, Pappone C, Mascioli G, Rossetti G et al. Risk stratification in Brugada syndrome. Results of the PRELUDE registry. *J Am Coll Cardiol* 2012;59:37-45.

16. Probst V VC, Eckardt L, Meregalli PG, Meregalli PG, Gaita F, Tan HL, Babuty D, Sacher F, Giustetto C, Schulze-Bahr E et al. Long-term prognosis of patients diagnosed with Brugada syndrome results from the FINGER Brugada Syndrome Registry. *Circulation* 2010;121:635-643.

17. Eckardt L, Probst V, Smits J, Bahr ES, Wolpert C, Schimpf R, Wichter T, Boisseau P, Heinecke A, Breithardt G et al. Long-term prognosis of individuals with right predordial ST-elevation-Brugada syndrome. *Heart Rhythm* 2005;2:S125.

18. Smits JP, Eckardt L, Probst V, Bezzina CR, Schott JJ, Remme CA, Haverkamp W, Breithardt G, Escande D, Schulze-Bahr E et al. Genotype-phenotype relationship in Brugada syndrome: electrocardiographic features differentiate SCN5A-related patients from non-SCN5A-related patients. *J Am Coll Cardiol* 2002;40:350-356.

19. Meregalli PG, Tan HL, Probst V, Koopmann TT, Tanck MW, Bhuiyan ZA, Sacher F, Kyndt F, Schott JJ, Albuisson J. Type of SCN5A mutation determines clinical severity and degree of conduction slowing in loss-of-function sodium channelopathies. *Heart Rhythm* 2009;6:341-348.

20. Gehi AK, Duong TD, Metz LD, Gomes JA, Mehta D. Risk stratification of individuals with the Brugada electrocardiogram: A meta-analysis. *J. Cardiovasc. Electrophysiol*. 2006;17:577-83.

21. Yamagata K, Horie M, Aiba T, Ogawa S, Aizawa Y, Ohe T, Yamagishi M, Makita N, Sakurada H, Tanaka T et al. Genotype-phenotype correlation of SCN5A mutation for the clinical and electrocardiographic characteristics of probands with Brugada syndrome: a Japanese multicenter registry. *Circulation* 2017;135:2255-2270.

22. Yang Y, Hu D, Sacher F, Kusano KF, Li X, Barajas-Martinez H, Hocini M, Li Y, Gao Y, Shang H et al. Meta-analysis of risk stratification of SCN5A with Brugada syndrome: is SCN5A always a marker of low risk? *Front. Physiol* 2019;10:103.

23. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E et al. ACMG Laboratory Quality Assurance Committee. 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 Med.* 2015;17:405-424.

24. Andorin A, Behr ER, Denjoy I, Crotti L, Dagradi F, Jesel L, Sacher F, Petit B, Mabo P, Maltret A et al. Impact of clinical and genetic findings on the management of young patients with Brugada syndrome. *Heart Rhythm* 2016;13:1274-1282.

25. Di Diego JM, Cordeiro JM, Goodrow RJ, Fish JM, Zygmunt AC, Pérez GJ, Scornik FS, Antzelevitch C. Ionic and cellular basis for the predominance of the Brugada syndrome phenotype in males. *Circulation* 2002;106:2004-2011.

26. Shimizu W, Matsuo K, Kokubo Y, Satomi K, Kurita T, Noda T, Nagaya N, Suyama K, Aihara N, Kamakura S et al. Sex hormone and gender difference—role of testosterone on male predominance in Brugada syndrome. *J. Cardiovasc. Electrophysiol.* 2007;18:415-421.

27. Ezaki K, Nakagawa M, Taniguchi Y, Nagano Y, Teshima Y, Yufu K, Takahashi N, Nomura T, Satoh F, Mimata H et al. Gender differences in the ST segment: effect of androgen-deprivation therapy and possible role of testosterone. *Circ J* 2010;74:2448-2454.

28. Song M, Helguera G, Eghbali M, Zhu N, Zarei MM, Olcese R, Toro L, Stefani E. Remodeling of Kv4.3 potassium channel gene expression under the control of sex hormones. *J. Biol. Chem.* 2001;276:31883-31890.

29. Vutthikraivit W, Rattanawong P, Putthapiban P, Sukhumthammarat W, Vathesatogkit P, Ngarmukos T, Thakkinstian A. Worldwide prevalence of Brugada syndrome: a systematic review and meta-analysis. *Acta Cardiol Sin* 2018;34:267-277.

30. Juang JJ, Horie M. Genetics of Brugada syndrome. *J Arrhythm*. 2016;32:418-425.

31. Bezzina CR, Shimizu W, Yang P, Koopmann TT, Tanck MW, Miyamoto Y, Kamakura S, Roden DM, Wilde AA. Common sodium channel promoter haplotype in asian subjects underlies variability in cardiac conduction. *Circulation* 2006;113:338-344.

32. Juang JJ, Liu YB, Chen CJ, Yu QY, Chattopadhyay A, Lin LY, Chen WJ, Yu CC, Huang HC, Ho LT et al. Validation and disease risk assessment of previously reported genome-wide genetic variants associated with Brugada syndrome: SADS-TW BrS Registry. *Circ Genom Precis Med.* 2020 Jun 3. doi: 10.1161/CIRCGEN.119.002797.

33. Makarawate P, Glinge C, Khongphatthanayothin A, Walsh R, Mauleekoonphairoj J, Amnueypol M, Prechawat S, Wongcharoen W, Krittayaphong R, Anannab A et al. Common and rare susceptibility genetic variants predisposing to Brugada syndrome in Thailand. *Heart Rhythm.* 2020;S1547-5271(20)30626-3. doi:10.1016/j.hrthm.2020.06.027

34. Robyns T, Nuyens D, Vandenberk B, Kuiperi C, Corveleyn A, Breckpot J, Garweg C, Ector J, Willems R. Genotype-phenotype relationship and risk stratification in loss-of-function SCN5A mutation carriers. *Ann Noninvasive Electrocardiol.* 2018;23:e12548.

Table 1. Demographics of the entire cohort (392 patients).

		Study group
Number of patients		392
Patient age at AE (year)	Age (mean±SD)	41.5 ± 14.7
	Patients<16 years	18 (4.6)
	16≥ Patients≤70	369 (94.1)
	Patients>70 years	5 (1.3)
Gender	Male	360 (91.8)
	Female	32 (8.2)
Ethnicity	White	205 (52.3)
	Asian	163 (41.6)
	Other/Unknown	24 (6.1)
Mode of AE documentation		
	Group A	267 (68.1)
	Group B	125 (31.9)
Family history of SCD		
	Yes	75 (19.1)
	No	303 (77.3)
	Unknown	14 (3.6)
History of syncope		
	Yes	139 (35.5)
	No	253 (64.5)
Presence of fever during AE		
	Yes	19 (4.9)
	No	306 (78)
	Unknown	67 (17.1)
Spontaneous type-1 BrS-ECG		
	Yes	260 (66.3)
	No	132 (33.7)
VF inducibility		
	EPS performed	219 (55.9)
	Inducible	136 (62.1)
	Not inducible	83 (37.9)

Abbreviations: Arrhythmic Event (AE); Sudden Cardiac Death (SCD); Brugada Syndrome Electrocardiogram (BrS-ECG); Ventricular Fibrillation (VF).

Table 2. The demographic, clinical, ECG, and EPS results of the study patients according to *SCN5A* genotype status: *P/LP* = pathogenic or likely pathogenic variant, *SCN5A* - = genotype negative.

		P/LP	*SCN5A* -	P value
Number of patients		44	300	
Patient age at AE (year)	Age (Mean±SD)	36.6±17.2	42.2±14	0.018
	Patients<16 years	5 (11.4)	9 (3.0)	0.023
	Patients≥16 years	39 (88.6)	291 (97.0)	
Gender				
	Male	36 (91.8)	281 (93.7)	0.013
	Female	8 (18.2)	19 (6.3)	
Ethnicity				
	White	35 (87.5)	134 (47.0)	<0.001
	Asian	5 (12.5)	151 (53.0)	
	Other/Unknown	4 (9.1)	15 (5.0)	
Mode of AE documentation				
	Group A	26 (59.1)	212 (70.7)	0.161
	Group B	18 (40.9)	88 (29.3)	
Family history of SCD				
	Yes	18 (41.9)	49 (16.8)	<0.001
	No	25 (58.1)	242 (83.2)	
	Unknown	1 (2.3)	9 (3.0)	1
History of syncope				
	Yes	17 (38.6)	102 (34.0)	0.611
	No	27 (61.4)	198 (66.0)	
Presence of fever during AE				
	Yes	4 (10.3)	11 (4.5)	0.137
	No	35 (89.7)	232 (95.5)	
	Unknown	5 (11.4)	57 (19.0)	0.294
Spontaneous type-1 BrS-ECG				
	Yes	30 (68.2)	197 (65.7)	0.865
	No	14 (31.8)	103 (34.3)	
VF inducibility				
	EPS performed	30 (68.2)	164 (54.7)	0.105
	Inducible	17 (56.7)	103 (62.8)	0.545
	Not inducible	13 (43.3)	61 (37.2)	

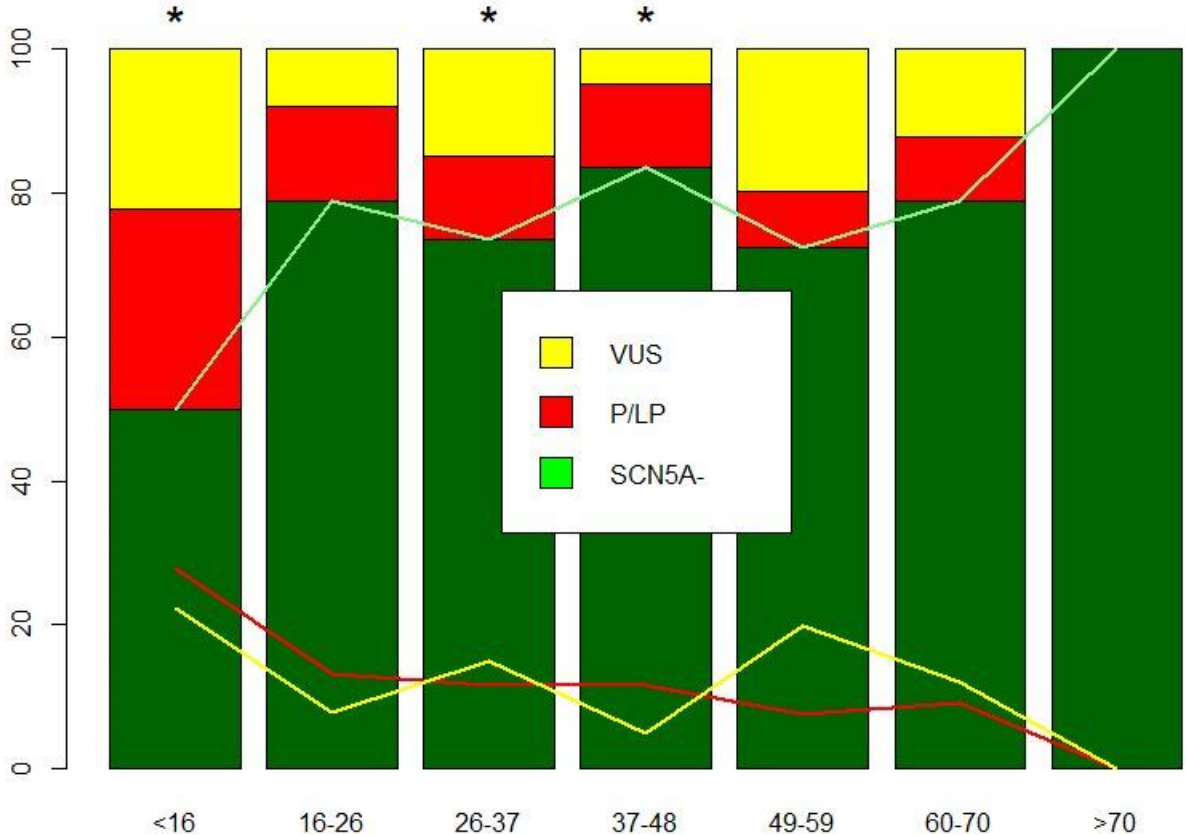
Abbreviations: as in Table 1

Table 3. Logistic regression of prediction of Pathogenic/likely pathogenic SCN5A variants vs. *SCN5A* negative.

Parameter	OR [95% CI]	p-value
Age at first AE<16 years	2.95 [0.76-10.74]	0.102
Male gender	0.59 [0.22-1.79]	0.328
Caucasian	5.09 [1.97-15.8]	0.002
Family Hx SCD	2.73 [1.28-5.82]	0.009

Figure legends

Figure 1. *SCN5A* genotype according to age group at time of AE. Relative proportions of *SCN5A* genotype according to age groups. P/LP = pathogenic and likely pathogenic; VUS = variant of uncertain significance; *SCN5A*- = genotype negative. Bars represent the relative proportion; straight line represent each colors proportion. * denotes a $p<0.05$ for fisher's exact comparison of each age group against the rest of the cohort.


*
*
*
100
80
60
40
20
0
VUS
P/LP
SCN5A-
<16
16-26
26-37
37-48
49-59
60-70
>70