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Clinical characteristics and electrophysiologic properties of *SCN5A* variants in fever-induced Brugada syndrome

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Summary

Background Brugada syndrome (BrS) is a severe inherited arrhythmia syndrome that can be unmasked by fever.

Methods A multicentre clinical analysis was performed in 261 patients diagnosed with fever-induced BrS, including 198 (75.9%) and 27 (10.3%) patients who received next-generation genetic sequencing and epicardial arrhythmogenic substrate (AS) mapping, respectively.

Findings In fever-induced BrS patients, pathogenic or likely pathogenic (P/LP) *SCN5A* variant carriers developed fever-induced BrS at a younger age, and more often in females and those of Caucasian descent. They exhibited significant electrophysical abnormalities, including a larger epicardial AS area, and more prolonged abnormal epicardial electrograms. During a median follow-up of 50.5 months (quartiles 32.5–81.5 months) after the diagnosis, major cardiac events (MCE) occurred in 27 (14.4%) patients. Patients with P/LP *SCN5A* variants had a higher ratio of MCE compared with the rest. Additionally, history of syncope, QRS duration, and Tpe interval could also predict an increased risk for future MCE according to univariate analysis. Multivariate analysis indicated that only P/LP *SCN5A* variants were

independent significant predictors of MCE. Computational structural modelling showed that most variants are destabilizing, suggesting that Nav1.5 structure destabilization caused by *SCN5A* missense variants may contribute to fever-induced BrS.

Interpretation In our cohort, P/LP *SCN5A* variant carriers with fever-induced BrS are more prevalent among patients of Caucasian descent, females, and younger patients. These patients exhibit aggressive electrophysiological abnormalities and worse outcome, which warrants closer monitoring and more urgent management of fever.

Keywords: Brugada syndrome; Fever; Genetics; Sudden cardiac death; Ventricular arrhythmias

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Research in context

Evidence before this study
Recent studies have shown that fever is able to induce or exacerbate the clinical presentation of BrS. Our previous study revealed that the prevalence of type 1 Brugada ECG pattern is nine times higher in subjects with fever, but the relationship between fever and type 2–3 Brugada ECG pattern is not significant. The objective of the present study is to describe the clinical features and unique characteristics of *SCN5A* variants contributing to the development of fever-induced BrS.

Added value of this study
The present study includes a large cohort of 261 fever-induced BrS patients to describe the clinical characteristics and the genotype–phenotype correlation. Fever-induced BrS patients with pathogenic/likely pathogenic (P/LP) *SCN5A* variants are more susceptible to fever-induced BrS at a younger age, exhibit a larger epicardial arrhythmogenic substrate area and more prolonged abnormal epicardial electrograms, and are more likely to have experienced major cardiac events during follow-up. Compared with patients of Asian descent, patients of Caucasian descent presented with fever-induced BrS at a younger age, are prone to have a higher yield of P/LP *SCN5A* variants, and more often have a family history of sudden cardiac death.

Implications of all the available evidence
Fever-induced BrS patients with P/LP *SCN5A* rare variants need more aggressive interventions, since they are more prone to presenting at younger age and with severe symptoms compared with those without. For risk stratification of fever-induced BrS, DNA sequencing and a systematic approach to the application of ACMG Guidelines for interpretation of *SCN5A* variants are critical.

Introduction

Brugada syndrome (BrS) is an inherited arrhythmia syndrome associated with an increased risk of sudden cardiac death (SCD) without gross structural abnormalities and characterized by the presence of a typical electrocardiographic (ECG) pattern consisting of an ST-segment elevation in the right precordial leads V1–V3. BrS patients are at risk for developing syncope, ventricular tachycardia (VT)/ventricular fibrillation (VF), or SCD. The mechanisms underlying the pathogenesis and development of arrhythmic events in BrS remain a matter of debate, but are believed to be due to either repolarization abnormality, depolarization abnormality, or both.[1–7] Abnormal expression of the neural crest on myocardial development of the right ventricular outflow tract (RVOT) and surrounding structures has also been suspected to play a fundamental role in the pathogenesis of BrS.[8] Catheter ablation over the anterior aspect of the RVOT epicardium has proven to be effective in reducing the manifestation of the BrS ECG pattern and control-

ling ventricular arrhythmias. Additionally, Nademanee et al. recorded fractionated electrogram activity and late potentials from the region and revealed that delayed depolarization over the anterior aspect of the RVOT epicardium contributes to BrS.[9] Variants in *SCN5A*, which encodes the cardiac sodium channel (Nav1.5), have been determined to be a pathogenic cause of the syndrome by using the ClinGen Gene-Disease Validity Classification Framework,[10] and comprise 20–30% of BrS cases with genetic screening.[11–13]

Several commonly recognized predisposing factors, including increased body temperature, drugs, and electrolyte disturbances, play a crucial role in unmasking the dynamic ECG changes of BrS.[14,15] In 1999, Dumaine et al. provided evidence that some loss-of-function of *SCN5A* mutations associated with BrS may be temperature-dependent, suggesting that this may underlie the ability of a febrile state to unmask the BrS phenotype.[16] However, fever-induced BrS is a clinically heterogeneous disease without a mutation-specific cause.[17] Recent studies have shown that a BrS pattern is much more prevalent (2–4%) in patients presenting in the emergency department with fever than in afebrile group of patients.[18,19] In the present study, we present the largest cohort to date of fever-induced BrS and report the clinical characteristics and outcomes of this cohort. Uniquely, we evaluate the *SCN5A* genetic substrate of fever-induced BrS and highlight the discrete arrhythmogenic substrate (AS) during epicardial mapping. Lastly, we evaluate the effect of these variants on the stability of the Nav1.5 structure in order to investigate potential molecular mechanisms of fever-induced BrS. Overall, our findings have implications for the risk stratification and management of patients associated with fever-induced BrS.

Methods

Ethics

This study was approved by the Institutional Review Board of each hospital and performed in accordance with the declaration of Helsinki. All participants gave written informed consent.

Clinical analysis and participants

Patients were collected from a multicentre registry. The study population consisted of 261 cases with fever-induced type 1 ECG at diagnosis (ST-segment elevation with type 1 morphology ≥2 mm in one or more right precordial leads in the second, third, or fourth intercostal space), all of whom meet the diagnostic criteria of BrS, including 198 patients who received next-generation genetic sequencing. Clinical follow-up was available for 188 patients. For comparison of the *SCN5A* variants between fever-induced and non-fever mediated BrS, 43 non-fever BrS cases carrying 47 *SCN5A* variants

selected from 243 patients in the above centers were enrolled as a control group. All these patients were diagnosed as BrS at the first visit to a hospital. The definition and diagnostic criteria were based on the Shanghai Score System for diagnosis of Brugada syndrome.[20] None of the subjects had a history of chronic heart disease (including ischemic heart disease, arrhythmia, valvular disease, and heart failure). Fever was defined as more than 100.4 °F (38 °C) measured in the bottom (rectally), 99.5 °F (37.5 °C) measured in the mouth (orally), and 99 °F (37.2 °C) measured under the arm (axillary). For each patient, we collected information including gender, age at diagnosis, race, clinical presentation, major cardiac events (MCE, including VT/VF or aborted SCD), history of syncope, family history of SCD, and the results of genetic screening.

Genetic screening and analysis

Next-generation sequencing for *SCN5A* gene variants was performed for each patient referred for genetic testing. Genomic DNA was extracted and purified from peripheral blood leukocytes using standard protocols. Genomic coordinates were based on the Array Tool (Agilent Technologies, Inc.), consisting of all isoforms present in the UCSC (http://genome.ucsc.edu/). The *SCN5A* variants are defined as any non-synonymous change to the wild-type sequence in all exons, such as missense variants, frameshift variants resulting from nucleotide insertions or deletions, and nonsense variants resulting from a termination codon. Besides, variants within either the first or the last 2 nucleotides in a particular exon, even if the nucleotide substitution code does not alter the open reading frame at all, have the capacity to alter proper mRNA splicing. As such, variants involving this exonic portion of the splice site were considered as possible splice-site variants in this study.

Genome Aggregation Database, ExAC, and 1000 genomes were applied to assess allele frequency of the variants found. Five *in silico* variant interpretation algorithms, including SIFT, PROVEAN (Protein Variation Effect Analyzer), Polyphen2, FATHMM, and CADD, were applied to estimate the pathogenicity of the genetic variants. Varsome platform (http://varsome.com/, accessed by March, 2022) was applied for ACMG classification to divide variants into pathogenic (P), likely pathogenic (LP), unknown significance (VUS), benign (B), and likely benign (LB).[21]

Swissprot database (http://ca.expasy.org/uniprot/) was applied to specify the topological placement of the variants by supplying the linear topologies for ion channels. The pore-forming alpha subunit structure of Nav1.5 is a large transmembrane protein consisting of 2016 amino acid residues. These residues form well-delineated domains and structural components: N-Terminal, four transmembrane-spanning regions (DI–DIV), three interdomain linkers (IDL of I–II, II–III, and

III–IV), and C-Terminal. Each transmembrane-spanning region was further segmented into S1 through S4, and the pore and channel selectivity filter of the channel (S5–S6).

Electrocardiogram measurements and epicardial mapping procedure

Each ECG record was independently interpreted by two experienced cardiologists from four major centres in China, United States, Italy, and The Netherlands, respectively. Then all ECGs and spreadsheet of related parameters were referred to Renmin Hospital of Wuhan University, China for further review and validation. Analysis of ECG wave forms included measurement of heart rate (HR), P-wave duration, PR interval, QRS duration, QT interval, corrected QT interval (Bazett's formula) in lead V5; and Tpeak-Tend (Tpe) interval in V1, V2, and V5. Among the study population, 27 patients underwent a combined endo-epicardial mapping procedure using a three-dimensional (3D) mapping system (CARTO 3, Biosense Webster, CA, USA) with a high-density mapping catheter (DecaNAV, Biosense Webster), according to the previously described technique.[22] All maps were obtained at baseline conditions and after drug challenge (ajmaline, up to 1 mg/kg). Ajmaline was administered to achieve the maximal ST-segment elevation and unmask, if necessary, the type 1 ECG pattern and to identify the whole extent of the regions displaying abnormal fragmented and delayed epicardial electrograms (EGMs), as previously described.[22,23] The potential duration map was created by collecting the duration of each EGM. As a result, a colour-coded map was obtained showing the regions displaying the shortest (red colour) and the longest (purple colour) durations, as previously described.[22,23] Arrhythmogenic substrate areas were measured and validated by three expert electrophysiologists using CARTO3 system.

Computational modelling of variant effect on $Na_v1.5$ structure

As protein stability is temperature dependent, proteins whose structures are destabilized by genetic variants have an increased tendency to unfold at elevated temperatures. Variant induced change in protein thermodynamic stability is often quantified by $\Delta\Delta G$, computed as the difference in Gibbs free energy of folding between the refined variant structure and the refined reference structure: $\Delta\Delta G_{reference \rightarrow variant} = \Delta G_{variant} - \Delta G_{reference}$. To investigate whether $Na_v1.5$ variants that linked to fever-induced BrS destabilize protein structure, we evaluated the $\Delta\Delta G$ of missense variants discovered in this work relative to the reference (wild-type) structure following a procedure described in a previous work.[24] Briefly, this procedure employed a computational $\Delta\Delta G$ protocol implemented in the

Rosetta biomacromolecular modelling software suite.[25,26] As a reference structure, we adopted the structural model of human Nav1.5 (UniProtKB accession number: Q14524, modelled residues: 30–440, 685–957, 1174–1887). This structural model covered 42 of the 75 functionally characterized missense variants (including 66 identified by our team and 9 reported by previous references). Recently, a cryo-EM structure of human Nav1.5 was determined at a resolution of 3.3 Å.[27] We compared our model with this experimentally determined structure and note that the root-mean-square distance between them over all backbone atoms is 2.3 Å, suggesting that our structural model is accurate.

Definitions of subgroups

Subgroup analysis was performed according to the different classification criteria. For analysis of the effect of the different variants on fever-induced BrS, the 198 patients referred for genetic testing were divided into four groups: (1) the *SCN5A*(+) group (patients with *SCN5A* variants; n = 77), (2) the *SCN5A*(–) group (patients without any variants, n = 121), (3) the P/LP *SCN5A*(+) subgroup (patients with *SCN5A* variants defined as P/LP according to ACMG classification; n = 59), (4) the B/LB/VUS *SCN5A*(+) subgroup (patients with *SCN5A* variants defined as B/LB/VUS according to ACMG classification, n = 18). Additionally, the entire 261 patients in the study were classified into two groups: MCE group (n = 32) and non-MCE group (asymptomatic patients or patients with syncope, n = 229). To investigate possible sex differences, analysis was performed for male (n = 210) and female (n = 51) subgroups. Finally, after the exclusion of six other or mixed race, the remaining 255 subjects were separated into two groups based on race: Asian group and Caucasian group (n = 101 and 154, respectively).

Statistical analysis

Continuous variables were expressed as mean ± standard deviation (SD) and evaluated using the Student t-test, one-way analysis of variance (ANOVA), and nonparametric statistics (Mann–Whitney U test/ Wilcoxon rank sum test and Kruskal–Wallis test). The distribution of data was tested by Shapiro–Wilk and Kolmogorov–Smirnov method. Descriptive statistics for categorical variables were presented as counts and percentages and assessed by the Chi-square test or Fisher's exact test. The Kaplan–Meier estimator was used to calculate time-to-major cardiac event distributions. Variables were included in the multivariate analysis if they had statistical significance in the univariate analysis. Collinearity analysis demonstrated no collinearity among the variables. Cox proportional hazards regression analysis was performed for multivariate comparisons. A P-value of <0.05 was considered statistically

Characteristic	Value (n = 261)
Gender	
Male, n (%)	210 (80.46)
Female, n (%)	51 (19.54)
Age (years)	41.73 ± 17.46
Proband, n (%)	241 (92.34)
Race	
Asian, n (%)	101 (38.70)
Caucasian, n (%)	154 (59.00)
Other, n (%)	6 (2.30)
Spontaneous type I Brugada ECG pattern during follow-up	120 (45.98)
Symptom	
Syncope, n (%)	61 (23.37)
VT/VF or aborted SCD, n (%)	32 (12.26)
Other, n (%)	27 (10.34)
Asymptomatic, n (%)	141 (54.02)
Proband with rare variants	
SCN5A(+), n (%)	77 (38.89)
P/LP SCN5A(+), n (%)	59 (29.80)
None, n (%)	121 (61.11)
Family history of SCD, n (%)	74 (28.35)
History of Syncope, n (%)	89 (34.10)
Positive drug test, n (%)	23/28 (82.14)
P/LP, pathogenic or likely pathogenic.	

***Table 1:* Characteristics of fever-induced BrS patients.**

significant. Statistical analysis was carried out using SPSS software version 21.0.

Results

Study population

The clinical characteristics of the patients are summarized in Table 1. A total of 261 patients (210 males and 51 females) were enrolled, showing a higher proportion of male subjects. The mean age at diagnosis of BrS was 41.73 ± 17.46 years. Of all patients, 154 were of Caucasian descent (59.00%), 101 were of Asian descent (38.70%), and six were of other or mixed descent (2.30%). In addition to all presenting with fever-induced type 1 BrS pattern, spontaneous type 1 BrS ECG pattern was present in 120 patients (45.98%) during follow-up (quartiles, 32.50–81.50 months). The symptoms were syncope in 61 (23.37%), VT/VF or aborted SCD in 32 (12.26%), other atypical symptoms (chest pain without lethal arrhythmia or primary infectious disease manifestations) in 27 (10.34%), 141 were asymptomatic (54.02%) at diagnosis and during the follow-up period. Of these patients, 74 (28.35%) had a family history of SCD, and 89 (34.10%) suffered from syncope before diagnosis. Multiple aetiologies of fever in these patients included not only infectious diseases, like upper respiratory tract infection, pneumonia, urinary tract infection, but also high ambient temperature (a hot bath in the jacuzzi) and vaccination.

Assessing *SCN5A* rare variants in fever-induced Brugada syndrome

To investigate the association between genetic variants and fever-induced BrS, DNA screening was performed in 198 patients. In 77 cases (38.89%), at least one *SCN5A* variant was identified (59 with P/LP variants). Together with nine previously reported variants identified in fever-induced BrS case reports, all variants are summarized in Supplementary Table S1. The type 1 BrS ECG pattern and its dynamic change observed in patients with *SCN5A* variants are illustrated in Fig. 1a–d. As shown in Table 2, clinical and ECG characteristics were compared between the P/LP *SCN5A* (+) group (patients with *SCN5A* variants defined as P/LP; n = 59) and the *SCN5A* (−) variant group (n = 121). First and foremost, among fever-induced BrS patients, carriers with P/LP *SCN5A* variants were significantly younger and had a significantly higher percentage of syncope before diagnosis than those in the *SCN5A* (−) variant group (32.62 ± 14.32 years *vs.* 44.61 ± 15.37 years, P < 0.001, Mann–Whitney U test; Fig. 2a; 50.85% *vs.* 33.06%, P = 0.022, Chi-square test). Additionally, the QT, QTc, PR, Tpe interval, and QRS duration were significantly longer in the P/LP *SCN5A*(+) group than in the *SCN5A* (−) variant group (380.39 ± 34.03 ms *vs.* 361.43 ± 50.69 ms, P = 0.001, Mann–Whitney U test; 421.52 ± 32.67 ms *vs.* 410.91 ± 48.16 ms, P = 0.004, Mann–Whitney U test; 180.20 ± 30.57 ms *vs.* 161.25 ± 28.13 ms, P = 0.001, Mann–Whitney U test; 91.60 ± 21.53 ms *vs.* 78.42 ± 23.09 ms, P = 0.001, Mann–Whitney U test; 115.75 ± 18.88 ms *vs.* 102.53 ± 25.43 ms, P = 0.004, Mann–Whitney U test; respectively). However, the differences persisted in the P/LP *SCN5A*(+) group compared with the *SCN5A*(−) group disappeared between the B/LB/VUS *SCN5A*(+) group (patients with *SCN5A* variants defined as B/LB/VUS, n = 18) and the *SCN5A*(−) group (Fig. 2a, Supplementary Table S2). The age difference comparing the *SCN5A*(−) group persisted when the *SCN5A*(+) group was divided by variant location and type (Fig. 2b and c).

Background characteristics of 27 (10.3%) patients who received epicardial AS mapping are summarized in Supplementary Table S2b. The size of the arrhythmogenic substrate and the duration of abnormal EGMs both at baseline and after ajmaline were significantly larger and more prolonged in patients harbouring a P/LP *SCN5A* variant (at baseline: 10.24 ± 2.31 cm² *SCN5A vs.* 5.88 ± 0.90 cm² non-*SCN5A*, P < 0.001, Student t-test; at baseline: 129.19 ± 10.78 ms in SCN5A patients *vs.* 105.03 ± 5.21 ms in non-*SCN5A* patients, P < 0.001, Mann–Whitney U test; after ajmaline: 20.27 ± 4.54 cm² *SCN5A vs.* 13.75 ± 1.73 cm² non-*SCN5A*, P = 0.002,
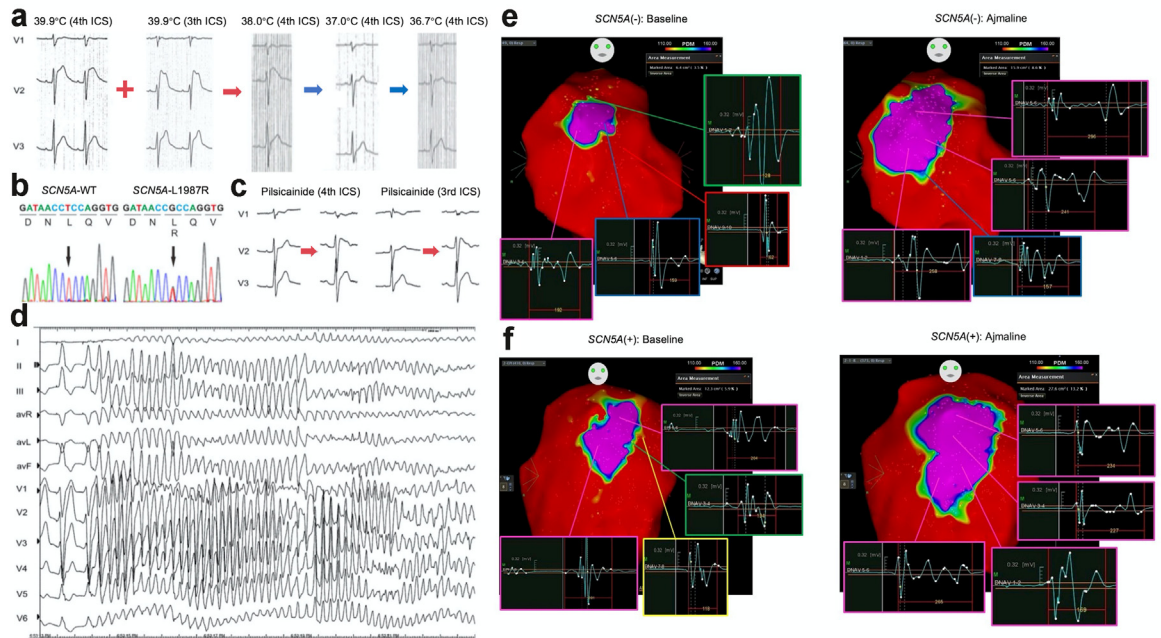
Fig. 1: Examples of fever-induced BrS patients with or without *SCN5A* rare variants. a–d: The 26-year-old male patient experienced recurrent syncopal episodes with mild upper respiratory symptoms and brief episodes of dyspnea. On admission, the body temperature was 39.9 °C and ECG showed marked ST elevation in leads V1–V3. Following antipyretic treatment, ECG changes normalized as body temperature decreased (**a**). Electropherogram showed the patient is heterozygous for CTC (Leu) and CGC (Arg) at codon (L1987R) (**b**). Pilsicainide unmasked a type 1 BrS ECG pattern (**c**). Programmed electrical stimulation at the RVOT induced polymorphic VT/VF (**d**). **e**: Male patient, 57 years old, a negative genetic test for *SCN5A* variants. History of recurrent syncope and fever-induced BrS type 1 pattern. During the follow-up, he experienced an appropriate ICD shock due to a VT/VF episode. The epicardial mapping demonstrated a 6.4 cm^2 area of abnormal electrograms (left panel), increasing to 15.9 cm^2 after ajmaline (right panel). **f**: Thirty-one-year-old male, *SCN5A* variant carrier, survived a previous cardiac arrest. Epicardial mapping demonstrated a large area (12.3 cm^2) of abnormal potentials (left panel), increasing to 27.6 cm^2 after ajmaline administration (right panel).

Student t-test; after ajmaline: 223.86 ± 13.10 ms in *SCN5A* patients *vs.* 201.00 ± 8.04 ms in non-*SCN5A* patients, P = 0.001, Student t-test; Table 2). Representative examples of the epicardial arrhythmogenic substrate results and epicardial EGMs are shown for patients with and without variants in the *SCN5A* gene (Fig. 1e and f).

The location of all variants in the *SCN5A*-encoded Nav1.5 pore-forming alpha subunit is shown in Fig. 3a. In all 80 *SCN5A* variants identified in the present study, most localized to the interdomain linkers (IDLs; 25.00%). Supplementary Tables S3 and S4 list the baseline and genetic characteristics of non-fever BrS patients with *SCN5A* variants. Compared with the control group (47 *SCN5A* variants identified in 43 non-fever *SCN5A*(+) BrS patients), a higher proportion of variants localizing to the IDLs were found in fever-induced BrS (25.00% *vs.* 10.64%, P = 0.049, Chi-square test) and no significant difference was found in the other domains (Fig. 3b). When B/LB/VUS *SCN5A*(+) variants were excluded, location difference between fever and non-fever BrS cases disappeared (Fig. 3c, Supplementary Table S4).

Risk stratification for fever-induced BrS
A comparison of clinical and ECG characteristics between the MCE group (n = 32) and the non-MCE group (asymptomatic patients or patients with syncope, n = 229) was carried out (Supplementary Table S5). Patients with documented MCE were significantly younger (35.33 ± 16.45 years *vs.* 42.62 ± 17.45 years, P = 0.027, Student t-test; Fig. 2d), and were comprised of a greater proportion of P/LP *SCN5A*(+) carriers and history of syncope (46.43% *vs.* 27.06%, P = 0.038, Chi-square test; 56.25% *vs.* 31.00%, P = 0.005, Chi-square test), and exhibited longer QRS duration and Tpe interval (117.68 ± 41.15 ms *vs.* 103.62 ± 17.42 ms, P = 0.029, Mann–Whitney U test; 88.56 ± 25.34 ms *vs.* 78.00 ± 21.43 ms, P = 0.018, Mann–Whitney U test).

During a median follow-up of 50.50 months after the diagnosis of fever-induced BrS, 27/188 (14.36%) patients suffered from MCE. Univariate analysis demonstrated that P/LP *SCN5A* variants, history of syncope, QRS duration ≥120 ms, and Tpe interval ≥100 ms were significant prognostic factors of fever-induced BrS (HR = 2.47, P = 0.019, Log–Rank test;
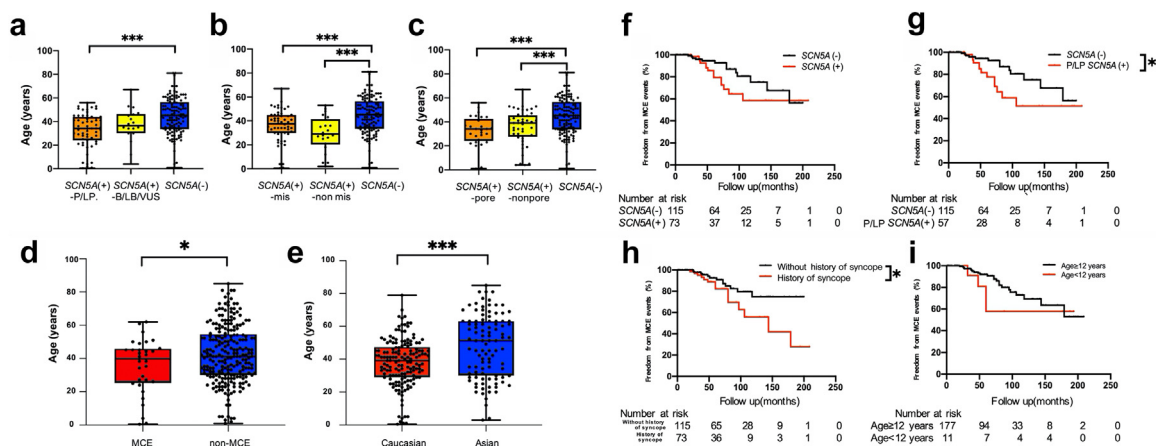
	SCN5A(−)	P/LP SCN5A(+)	P
Clinical characteristics	(n = 121)	(n = 59)	
Male, n (%)	99 (81.82)	36 (61.02)	0.002[a]
Age (years)	44.61 ± 15.37	32.62 ± 14.32	<0.001[a]
Proband, n (%)	111 (91.74)	49 (83.05)	0.082
History of syncope, n (%)	40 (33.06)	30 (50.85)	0.022[a]
MCE, n (%)	14 (11.57)	13 (22.03)	0.065
Family history of SCD, n (%)	35 (28.93)	22 (37.29)	0.258
Asian, n (%)	28 (23.14)	4 (6.78)	0.007[a]
Caucasian, n (%)	91 (75.21)	54 (91.53)	0.009[a]
ECG parameters	(n = 121)	(n = 59)	
HR (bpm)	81.17 ± 17.71	77.98 ± 16.73	0.411
QT interval in V5 (ms)	361.43 ± 50.69	380.39 ± 34.03	0.001[a]
QTc interval in V5 (ms)	410.91 ± 48.16	421.52 ± 32.67	0.004[a]
QRS duration in V5 (ms)	102.53 ± 25.43	115.75 ± 18.88	0.001[a]
PR interval in V5 (ms)	161.25 ± 28.13	180.20 ± 30.57	0.001[a]
Tpe interval in V1 (ms)	78.42 ± 23.09	91.60 ± 21.53	0.001[a]
Epicardial arrhythmogenic substrate characteristics	(n = 6)	(n = 20)	
Baseline substrate size (cm^2)	5.88 ± 0.90	10.24 ± 2.31	<0.001[a]
Substrate size after ajmaline (cm^2)	13.75 ± 1.73	20.27 ± 4.54	0.002[a]
Baseline potential duration (ms)	105.03 ± 5.21	129.19 ± 10.78	<0.001[a]
Potential duration after ajmaline (ms)	201.00 ± 8.04	223.86 ± 13.10	0.001[a]

P/LP, pathogenic or likely pathogenic. [a]Indicates P < 0.05 between two groups.

Table 2: Clinical characteristics of different *SCN5A* rare variant carriers with fever-induced BrS.

HR = 2.29, P = 0.033, Log–Rank test; HR = 2.36, P = 0.032, Log–Rank test; HR = 2.56, P = 0.023, Log–Rank test; Fig. 2f–i, Table 3). Nevertheless, multivariate Cox regression analysis performed by using the above significant prognostic factors indicated that only the P/LP *SCN5A* variant was an independent significant predictor of MCE (HR = 2.31; P = 0.031, Log–Rank test).

Fig. 2: Comparison of age at diagnosis and Kaplan–Meier analysis of the MCE–free survival in fever-induced BrS. a: Box plot of age at diagnosis among P/LP *SCN5A*(+), B/LB/VUS *SCN5A*(+) and *SCN5A*(−) groups (Mann–Whitney U test). **b**: The correlation between age and type of *SCN5A* variants. "mis/non mis" indicates missense/non-missense variants (Mann–Whitney U test). **c**: The correlation between age and location of *SCN5A* variants. "pore/nonpore" indicates variant in pore/non-pore region (Student t-test). **d**: Age at diagnosis between MCE and non-MCE groups (Student t-test). **e**: Age at diagnosis between groups of Caucasian and Asian descent (Mann–Whitney U test). **f**: Analyses comparing patients with and without *SCN5A* variants [*SCN5A*(+) *vs.* *SCN5A*(−)] (Log–Rank test). **g**: Analyses comparing patients with P/LP SCN5A(+) variants and without *SCN5A* variants [P/LP *SCN5A*(+) *vs.* *SCN5A*(−)] (Log–Rank test). **h**: Analyses comparing patients according to with and without the history of syncope (Log–Rank test). **i**: Analyses comparing patients according to age at diagnosis. P/LP, pathogenic or likely pathogenic; B/LB/VUS, benign, likely benign, or unknown significance. MCE, major cardiac events (Log–Rank test). * indicates P < 0.05 between two groups, *** indicates P < 0.001 between two groups.
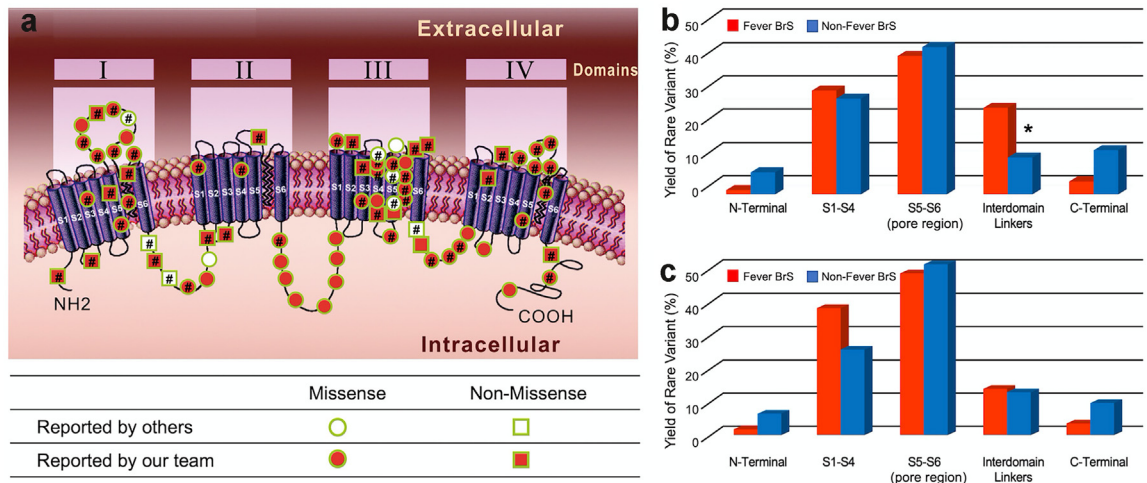
Fig. 3: Topology of Nav1.5 and Location of Variants Identified in Fever-induced BrS as well as Differences in Frequency of Variants Localizing to Specific Regions of the Nav1.5 in Fever-induced and Non-fever BrS. a: Topology of Nav1.5 and location of rare variants identified in fever-induced BrS. Missense variants are depicted by circles, and non-missense variants (including frameshift, splice-site, non-sense) are depicted by squares. Variants identified in this study are depicted with red symbols, whereas those previously published are depicted by white symbols. #, variants defined as pathogenic/likely pathogenic according to ACMG (See details in Supplementary Table S1). **b and c:** Differences in localizing frequency of the Nav1.5 rare variants identified in fever-induced and non-fever BrS. Red and blue histograms depict the fever-induced and non-fever-induced BrS controls, and the vertical axis represents the frequency of variants localizing to specific regions of the Nav1.5. **Panel b** includes all variants from this study. **Panel c** includes variants with pathogenic/likely pathogenic classification from this study. * indicates $P < 0.05$ (Chi-square test).

Race, gender, and fever-induced BrS

There were four principal differences between patients of Asian or Caucasian descent (n = 101 and 154, respectively) concerning fever-induced BrS (Supplementary Table S6). First, the proportion of males of Asian descent was higher than those of Caucasian descent (93.07% *vs.* 72.08%, P < 0.001, Chi-square test). Second, we noted a larger proportion of patients of Caucasian descent with P/LP variants and family history of SCD (35.29% *vs.* 10.26%, P = 0.002, Chi-square test; 33.12% *vs.* 20.79%, P = 0.032, Chi-square test). Third, the average age of patients of Asian descent was significantly higher (47.82 ± 19.47 years *vs.* 37.95 ± 14.51 years, P < 0.001, Mann–Whitney U test; Fig. 2e). Finally, ECGs recorded from patients of Caucasian descent displayed significantly longer QT interval, QRS duration, and Tpe interval compared with patients of Asian descent (372.93 ± 46.55 ms *vs.* 351.19 ± 46.30 ms, P < 0.001, Mann–Whitney U test; 109.77 ± 25.12 ms *vs.* 98.70 ± 14.18 ms, P < 0.001, Mann–Whitney U test; 84.01 ± 23.26 ms *vs.* 71.76 ± 18.09 ms, P < 0.001, Mann–Whitney U test). With respect to the gender comparison (male/female ratio: 4.12), higher proportions of patients of Caucasian descent and *SCN5A* variant carriers were observed in the female population (84.31% *vs.* 52.86%, P < 0.001, Chi-square test; 54.17% *vs.* 34.00%, P = 0.013, Chi-square test; Supplementary Table S7).

Computational modelling indicates that most variants are destabilizing

The stability of protein structures is temperature dependent. Proteins whose structures are destabilized by genetic variants have an increased tendency to unfold, thus may lose functionality, at elevated temperatures. We thus hypothesized that destabilization of Na$_v$1.5 structure could contribute to fever-induced BrS. Our computational ΔΔG calculations are summarized in Fig. 4. Out of the 42 variants mapped to the structural model, 19 *SCN5A* variants (D197Y, G292S, L325R, L382F, S805L, E1225K, D1275N, V1340I, F1344S, V1353M, L1412F, R1512Q, R1512W, A1529D, L1582P, V1667D, F1697L, M1701R, and G1743E) are predicted to have an appreciable destabilization effect on Nav1.5 structure (ΔΔG ≥ 1 kcal/mol). For a protein with typical thermodynamic stability (ΔG = −5 kcal/mol), a destabilization by 1 kcal/mol (ΔΔG = 1 kcal/mol) will reduce the equilibrium constant for the folding reaction of this protein by a factor of 5.4 at room temperature (293K). Six additional variants (Y314D, D356N, R1193Q, R1306C, G1406R, and L1501V) are predicted to have a destabilizing influence, although with smaller effects (ΔΔG within 0.5–1 kcal/mol). Out of the remaining variants, V263I, L1308F, L1373S, V1429M, T1620M, and D1792N are predicted to be moderately stabilizing (ΔΔG within −0.5 to −1 kcal/mol), while R282H, L306F, T1304M, and R1306H shows a very minor destabilizing effect, and A344S, D349N, D1370G, T1709M, and

	MCE (n = 27)	non-MCE (n = 161)	Analysis	Univariate analysis		Multivariate analysis	
				HR (95% CI)	P	HR (95% CI)	P
Clinical characteristics							
Male, n (%)	23 (85.19)	119 (73.91)	Yes vs. No	0.58 (0.20–1.67)	0.311		
Age < 12 years, n (%)	4 (14.81)	7 (4.35)	Yes vs. No	1.56 (0.53–4.63)	0.421		
History of syncope, n (%)	14 (51.85)	59 (36.65)	Yes vs. No	2.29 (1.07–4.90)	0.033[a]	2.13 (0.99–4.59)	0.053
Family history of SCD, n (%)	9 (33.33)	49 (30.43)	Yes vs. No	0.95 (0.42–2.11)	0.899		
Caucasian, n (%)	21 (77.78)	125 (77.64)	Yes vs. No	1.20 (0.48–2.99)	0.696		
Genetic characteristics							
Genotype							
SCN5A(+), n (%)	14 (51.85)	59 (36.65)	Yes vs. No	1.93 (0.91–4.11)	0.089		
P/LP SCN5A(+), n (%)	13 (48.15)	44 (27.33)	Yes vs. No	2.47 (1.16–5.27)	0.019[a]	2.31 (1.08–4.94)	0.031[a]
Variant type							
Missense, n (%)	10 (37.04)	42 (26.09)	vs. non-variants	2.02 (0.81–5.04)	0.086		
Non-missense, n (%)	3 (11.11)	17 (10.56)	vs. non-variants	1.28 (0.33–4.97)	0.701		
ACMG							
P/LP SCN5A(+), n (%)	13 (48.15)	44 (27.33)	vs. non-variants	2.33 (1.01–5.40)	0.026[a]		
B/LB/VUS SCN5A(+), n (%)	1 (3.70)	15 (9.32)	vs. non-variants	0.60 (0.12–3.11)	0.614		
Location							
Pore, n (%)	6 (22.22)	21 (13.04)	vs. non-variants	2.08 (0.65–6.68)	0.127		
Non-pore, n (%)	6 (22.22)	37 (22.98)	vs. non-variants	1.48 (0.51–4.26)	0.421		
ECG characteristics							
HR ≥ 100 bpm in V5, n (%)	1 (3.70)	18 (11.18)	Yes vs. No	0.20 (0.03–1.46)	0.112		
PR ≥ 200 ms in V5, n (%)	5 (18.52)	20 (12.42)	Yes vs. No	1.86 (0.70–4.97)	0.217		
QRS ≥ 120 ms in V5, n (%)	10 (37.04)	34 (21.12)	Yes vs. No	2.36 (1.07–5.17)	0.032[a]	1.87 (0.83–4.22)	0.131
QTc ≥ 460 ms in V5, n (%)	2 (7.41)	14 (8.70)	Yes vs. No	0.50 (0.12–2.12)	0.345		
Tpe ≥ 100 ms in V1, n (%)	9 (33.33)	37 (22.98)	Yes vs. No	2.56 (1.14–5.76)	0.023[a]	1.53 (0.60–3.87)	0.373

P/LP, pathogenic or likely pathogenic; B/LB/VUS, benign, likely benign, or unknown significance. [a]Indicates $P < 0.05$ between two groups.

*Table 3*: Univariate and multivariate analysis of major cardiac events in fever-induced BrS.

S1710L almost have no effect on stability ($\Delta\Delta G \approx 0$ kcal/mol).

Discussion

This study provides a comprehensive assessment of clinical characteristics of 261 patients aimed at identifying the uniqueness of fever-induced BrS (Fig. 5). Among all subjects enrolled, 93 developed typical symptoms (61 syncope and 32 VT/VF or aborted SCD) related to the BrS at diagnosis or during follow-up, which appeared to challenge the current view that fever-induced BrS is relatively more benign than spontaneous ones. It has previously been observed that fever might result in a twenty-fold increase in the prevalence of BrS in the general population, but these patients with fever-induced type 1 BrS ECGs are asymptomatic and remain so during follow-up.[18] Nevertheless, the fact that fever may be a trigger for arrhythmic events not only in children but also in adults has been identified in several studies.[28] The survey based on SABRUS has revealed that approximately 6% of arrhythmic events in BrS are associated with fever.[29] Unfortunately, the proportion of fever-induced BrS without arrhythmic events remains largely unknown in their research, which makes it difficult to evaluate the adverse prognosis of fever-

induced BrS. However, similar to our study, they report that young BrS cases (age < 16 years) comprise a considerable proportion (37.10%) of fever-related arrhythmic events, which is believed to be due to the relatively high prevalence of SCN5A rare variants and susceptibility to febrile disease.

Although it is well-known that the genetic defect in SCN5A leads to the BrS phenotype, the prognostic value is ambiguous because of the lack of conclusive studies. By using the heterologous expression system and whole-cell patch clamping technique, Ishikawa, et al. found that BrS patients with functionally proven loss-of-function SCN5A mutations are more prone to sudden death.[30] Similarly, in our previous studies, we have observed that the rate of life-threatening events is significantly higher in the individuals with P/LP SCN5A variants based on the ACMG criteria.[22] A recent study of 415 probands in a Japanese multicentre registry display that the SCN5A mutations are genetic burdens of cardiac events in BrS, especially those located in the pore region of Nav1.5.[31] However, in the present study on fever-induced BrS, the incidence of MCE does not appear to be affected by the location or type of variants. After the ACMG criteria screening, our patients with P/LP variants tended to experience more MCEs compared
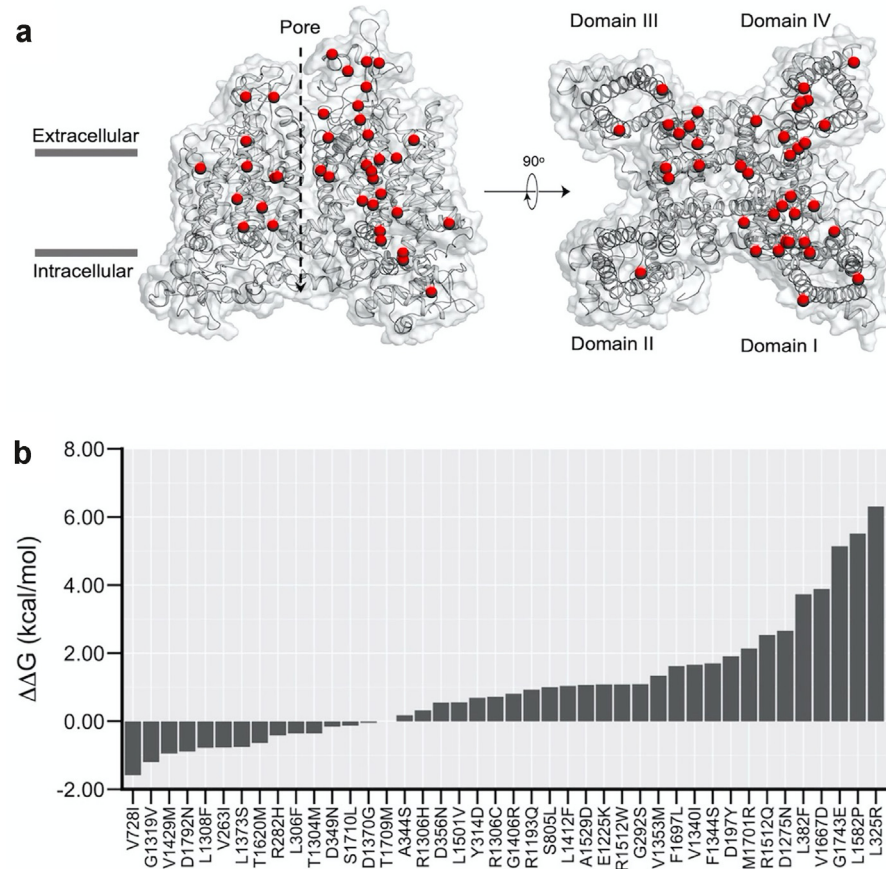
Fig. 4: Computational modelling on structural stability of the Nav1.5 rare variants identified in fever-induced BrS. **a**: Forty-two out of the seventy-five *SCN5A* variants in this study can be mapped to a structural model of Nav1.5. The shape of the Nav1.5 structural model is surface-rendered, and its structural components are represented by helical cartoons. The locations of the forty-two variants in the model are represented by red spheres. **b**: Changes in thermodynamic stability (ΔΔG) of Nav1.5 caused by the 42 variants mapped to the structural model were calculated using a computational protocol implemented in the Rosetta software suite. Twenty-five out of these 42 variants were predicted to be destabilizing (ΔΔG > 0.5 kcal/mol), while eight were predicted to be stabilizing (ΔΔG < –0.5 kcal/mol). The remaining nine variants were predicted to have little effect (ΔΔG within –0.5 to 0.5 kcal/mol) on Nav1.5 stability.

with patients without *SCN5A* rare variants during follow-up. Also, several ECG parameter discrepancies identified in BrS have previously been proven conducive to identifying patients at higher risk of MCE, such as S wave width and ST elevation in V1 or V2, Tpe interval, and QRS interval.[32,33] Our study further confirms the predictive value of relatively longer QRS and Tpe interval caused by P/LP *SCN5A* variants in fever-induced BrS, while the potential role of other markers may be validated by larger sample sizes in future studies.

Similar to prior reports, most of the patients in our study are male, and they are approaching middle age at the time of the first diagnosis. The mean age of the *SCN5A* group, however, is significantly lower than that of the non-*SCN5A* group. The proportion of *SCN5A* variant carriers in young BrS patients (age < 17 years) is much higher (approximately 62%–77%).[34] In our

previous studies concerning Brugada-like syndrome in infants, this percentage is similar (3 *SCN5A* variants and 2 *CACNB2b* variants identified in 5 cases).[35] A recent study has also reported that, for both children and adolescents, *SCN5A* variant carriers are more prone to develop fever-induced type-1 BrS ECG pattern.[36] It is noteworthy that young patients (age < 17 years) make up only 3.2% of people known to have BrS.[37] Our data reports on the genetic background of *SCN5A* among all age groups, further demonstrating the relevance of *SCN5A* variants in younger patients, especially in patients of Caucasian descent and patients with MCE, which are absent in reported non-fever BrS. Therefore, in the setting of fever, one may expect an important role for the *SCN5A* variants in younger fever-induced BrS subjects and related to severe outcome. A recent meta-analysis of common variants associated with atrial and
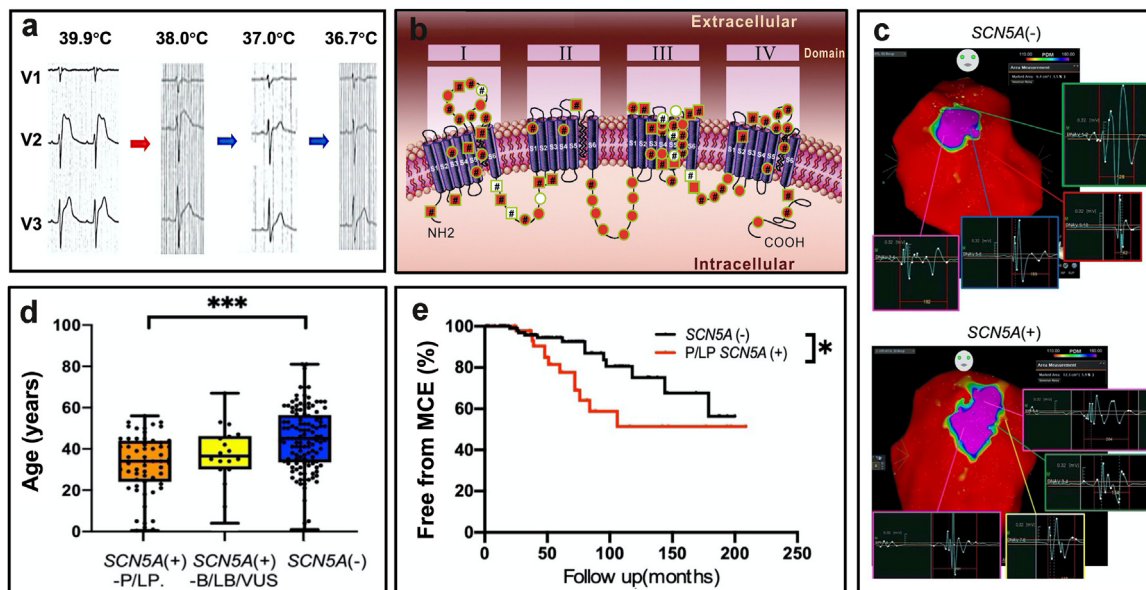
Fig. 5: Graphical Abstract of Fever-Induced Brugada Syndrome. a: Exemplary case of the *SCN5A* carrier with fever-induced type 1 ECG. b: Location of the Nav1.5 rare variants identified in fever-induced BrS. Variants identified in this study are depicted with red symbols whereas those previously published are depicted by white symbols. #, variants defined as pathogenic/likely pathogenic according to ACMG. c: Arrhythmogenic substrate characterization according to the presence or absence of *SCN5A* rare variants by the epicardial mapping. d: Differences of age at diagnosis in fever-induced BrS (Mann–Whitney U test). e: Kaplan–Meier analysis of the MCE–free survival for fever-induced BrS (Log–Rank test). P/LP, pathogenic or likely pathogenic; B/LB/VUS, benign, likely benign, or unknown significance. MCE, major cardiac events.

atrioventricular electrical activity also concludes that *SCN5A* is significantly associated with the PR interval.[38] In the present scenario, the significant difference between the *SCN5A* group and the non-*SCN5A* group in PR interval still exists.

After initial reports of electrophysiological abnormalities in the epicardium of the right ventricle in BrS patients, it has been demonstrated that these electrophysiological abnormalities are mechanistically responsible for the type 1 BrS ECG pattern, and their successful elimination by catheter ablation may result in the abolition of the BrS pattern and suppression of ventricular arrhythmias.[5,22,23] In this study, patients harbouring *SCN5A* mutations showed more aggressive electrophysiological epicardial abnormalities as compared to those without *SCN5A* mutations, which may explain why these patients have a more severe clinical presentation, more frequently experiencing cardiac arrest or ventricular arrhythmias.[22,31] Recently, it has been demonstrated that the genetic status contributes to the electrophysiological phenotypic expression of the disease, being associated with a larger area of epicardial abnormalities providing an increased risk of life-threatening arrhythmias.[22] This suggests a prognostic implication of *SCN5A* variants when evaluating a patient with BrS.

On the question of age at the time of diagnosis, the SABRUS population-based studies show no significant difference in the average age for onset of arrhythmic events in patients of Caucasian and Asian descent.[39] When the study subjects are limited to those with fever-related arrhythmic events, patients of Asian descent are much older at the time of onset of arrhythmic events.[29] However, patients of Caucasian descent present with less aborted cardiac arrest in comparison to patients of Asian descent.[29,39] According to our results, patients of Caucasian descent with *SCN5A* rare variants are more easily detected in childhood or youth, which may partially explain differences in symptoms, races, ages, and electrophysiology parameters. Multiple studies have exhibited a male predominance in BrS, which is explained by differences in testosterone levels and ion channel function.[40] Although the male predominance is maintained in our population, with an even larger proportion of males in the group of Asian descent, there is a significant difference in the sex ratio between the groups of Asian and Caucasian descent. Whether the discrepancy concerning fever susceptibility or estrogen protection exists between females of Asian and Caucasian descent awaits further investigation. Additionally, there were no observable sex differences in clinical symptoms, which appeared to

differ from the conventional wisdom that males with BrS present with greater risk and have a worse prognosis.[40] Similar to a recent study about gender differences in BrS cases,[41] females with fever-induced BrS have a higher *SCN5A* variant yield, likely countering the effects of estrogen protection in females.

Understanding of the molecular mechanism underlying fever-induced BrS is currently incomplete and multiple contributing factors have been suggested.[18] Patch-clamp studies by Dumaine et al. at elevated temperature (32 °C) showed that the T1620M variant had faster current decay kinetics when compared with the wild type and that steady-state activation was significantly shifted.[16] In contrast, these differences between T1620M and the wild type were not observed at room temperature (22 °C). These results suggest that mutations can increase the temperature sensitivity of several biophysical properties of Nav1.5. However, another study by Keller et al. on the L325R variant suggests that mutations with severe or complete loss of function at physiological temperature may unmask the temperature-sensitivity of wild-type channel at elevated temperature through a dominant-negative effect.[17] Here, using computational structural modelling we estimated that a majority of variants decreased the stability of Nav1.5 structure, which suggest that loss of stability could be a contributing factor to fever-induced BrS especially considering the fact that proteins tend to unfold at higher temperatures. In fact, we estimated that L325R had a significantly lower stability than the wild type ($\Delta\Delta G = 3.94$ kcal/mol). This is consistent with the observation that L325R is misfolded and that its I_{Na} peak current can be partially rescued when the variant was expressed at a lower temperature (28 °C).[17]

Limitation and conclusion

Several limitations pertain to our study. In this study, approximately 10% of the study population underwent epicardial mapping to characterize the arrhythmogenic substrate. This was a subset of patients with various clinical characteristics, evaluated and/or referred to an experienced BrS center, because they showed an increased arrhythmic risk profile. Therefore, these results might not be applicable to all other patient populations. Since many single nucleotide polymorphisms (SNPs) are reported or established associations with the phenotype of fever-induced BrS for the first time, most are classified as variants of "uncertain" significance according to ACMG guidelines. Future studies would provide additional evidence to support the association of particular variants to the disease. Although functional analysis of the genetic variants is limited, the computer modelling employed provides a potential mechanism. As expected from our previous study of Nav1.5 variants,[24] this analysis shows that not all BrS-associated *SCN5A* variants are destabilizing. However, the fact that most of the variants are destabilizing suggests that Nav1.5 structure destabilization likely contributes to variant pathogenicity in fever-induced BrS. In addition, while our computational modelling shows that a majority of variants are destabilizing, we currently are not able to conclude whether loss of stability is a causal molecular mechanism under fever-induced BrS. Ideally, to better establish the association between structure destabilization and fever-induced BrS, we would also need a set of *SCN5A* variants from BrS patients whose clinical presentations are not affected or unmasked by fever. We could then compute the $\Delta\Delta G$s of this set of variants using the exact same computational modelling procedure. If loss of stability is a causal molecular mechanism, we could expect this set of variants to a smaller $\Delta\Delta G$ (less destabilizing) than variants associated with fever-induced BrS on average. This could be an important investigation in the future when relevant variant data becomes available.

In summary, the present study investigates one of the largest cohorts of fever-induced BrS patients to describe the clinical characteristics and the genotype–phenotype correlation. It indicates that the P/LP *SCN5A* rare variants are closely associated with fever-induced BrS, especially among patients of Caucasian descent, female patients, and younger patients. These variants are correlated with more aggressive electrophysiological abnormalities and real clinical risk during follow-up, which warrants closer monitoring, more aggressive genetic testing, and more urgent management of fever.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2022.104388.

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