Utilizing Multiple in Silico Analyses to Identify Putative Causal *SCN5A* Variants in Brugada Syndrome

Jyh-Ming Jimmy Juang,[1,2] Tzu-Pin Lu,[3] Liang-Chuan Lai,[2] Chia-Hsiang Hsueh,[4] Yen-Bin Liu,[1] Chia-Ti Tsai,[1] Lian-Yu Lin,[1] Chih-Chieh Yu,[1] Juey-Jen Hwang,[1] Fu-Tien Chiang,[1] Sherri Shih-Fan Yeh,[5] Wen-Pin Chen,[6] Eric Y. Chuang[3,7], Ling-Ping Lai,[1] Jiunn-Lee Lin[1]

[1]Division of Cardiology, Department of Internal Medicine, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan

[2]Graduate Institute of Physiology, College of Medicine, National Taiwan University, Taipei, Taiwan

[3]YongLin Biomedical Engineering Center, National Taiwan University, Taipei, Taiwan

[4]Department of Medicine, Krannert Institute of Cardiology and Division of Cardiology, Indiana University School of Medicine, Indianapolis, Indiana

[5] Department of Environmental and Occupational Medicine, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan

[6]Institute of Pharmacology, College of Medicine, National Taiwan University, Taipei, Taiwan

[7]Graduate Institute of Biomedical Electronics and Bioinformatics, National Taiwan University, Taipei, Taiwan

Supplementary Methods

Study subjects

Symptomatic BrS is definitively diagnosed according to the current consensus when a Type I Brugada ECG is observed in more than one right precordial lead (V1-V3), in the presence or absence of sodium channel blocking agent, and in conjunction with one or more of the following: documented ventricular fibrillation (Vf); polymorphic ventricular tachycardia (VT); a family history of sudden cardiac death (< 45 years old); coved-type ECGs in family members; inducibility of VT with programmed electrical stimulation (PES); syncope; or nocturnal agonal respiration [1-4]. Five hundred fifty-one healthy individuals with no cardiac arrhythmia and normal ECG were genotyped as a control group.

In silico functional analyses of the sequence alterations in *SCN5A*

SIFT

The homologous sequences were selected according to the following parameters: (1) UniProt-TrEMBL 39.6 was used as reference protein database; (2) the median conservation score was set as 3.0; (3) sequences more than 90% identical to the query sequence were removed.

PolyPhen-2

We used the default settings in PolyPhen-2 to calculate probability scores for each pair of altered variants (e.g., H558R) in SCN5A. The higher the probability score, the higher the functional impact a specific non-synonymous substitution is likely to have.

Align-GVGD

The GV score measures the degree of biochemical variations observed in a particular locus in the alignment, and the GD score estimates the biochemical differences between a pair of altered and wild type variants. SCN5A variants that were more likely to interfere with protein function were grouped into a class with larger numbers.

Validating the computed results using mass spectrometric technology in a large healthy population

The genotypes of the five targeting loci were assessed according to the iPLEX protocol by using the MassARRAY system (Sequenom, San Diego, CA). The primers for PCR were designed based on the SeqTool Document v1.0 (Institute of Biomedical Sciences, Sinica, Taipei, Taiwan), and MassARRAY TyperAnalyzer v3.3 software provided by Sequenom was utilized to determine the genotyping results.

Cloning of *SCN5A* and *SCN1B* and generation of *SCN5A* variant constructs

Complimentary DNAs were synthesized from total RNA, which were extracted from human heart tissue, using the Superscript III reverse transcription kit (Invitrogen, USA), and the reaction product was used as the template in subsequent PCR. To make the co-expression system mimic to human cardiac sodium channel, the PCR product of *SCN1B that is an important subunit of cardiac sodium channel* was first cloned into pAAV-IRES-hrGFP (Stratagene, USA) with *Bam*H I/*Xho* I and subcloned into the *Bgl* II recognition sequence of pBudCE4.1 (Invitrogen) with *Bam*H I/*Bgl* II. The subcloning procedure allowed dicistronic expression of SCN1B and

humanized *Renilla reniformis* green fluorescent protein (hrGFP) under the control of the EF-1 promoter. The PCR product of *SCN5A* was then cloned into the pBudCE4.1 vector containing *SCN1B* with *Hin*d III/*Xba* I and the CMV promoter (Figure S1). The base sequences of the *SCN5A* and *SCN1B* clones were compared to the sequences of the published *SCN5A* (hH1, NM_198056) and *SCN1B* (NM_199037) clones. The mutated constructs of the loci with high probability in functional dysregulationswere generated using the QuickChange Site-Directed Mutagenesis system (Stratagene). All constructs were sequenced to verify the mutations and to rule out possible PCR errors.

Cell culture and transfection of *SCN5A* variant constructs

HEK293T cells (10^5) were seeded into 12-well plates the day before transfection and grown in DMEM supplemented with 10% FBS and antibiotics at 37°C and 5% CO_2. One μg vector of *SCN5A* mutants and 2 μL JET-PEI (Polyplus,) in 100 μL NaCl (150 mM) solution were added into the cell monolayer. The cells were trypsinized for patch clamp 48 h after transfection.

The procedures and details of measuring steady-state activation and inactivation

Steady-state activation was studied by measuring the peak sodium conductance (G_{Na}) during a 45 msec test pulse to various test potentials from −80 mV holding voltage. G_{Na} was calculated from $G_{Na} = I$ hNav1.5/($V - V_{rev}$), where I hNav1.5 is the peak sodium current during the test depolarization (V), and V_{rev} is the sodium reversal potential. Data were normalized to maximum peak conductance (G_{max}) and fitted to a Boltzmann distribution: G_{Na}/G_{max} = (1 +

$\exp[(V - V_{0.5})/s_{,act}])^{-1}$, where $V_{0.5}$ is the potential for half-maximal activation and $s_{,act}$ is the slope of the activation curve.

To study steady-state fast inactivation, cells were held at prepulse potentials ranging from −140 to -30 mV for 3 sec and then subjected to a -10 mV test pulse for 25 msec [5]. Normalized peak currents were plotted versus prepulse potentials, and curves were fitted by the Boltzmann function: $I/I_{max} = (1 + \exp[(V - V_{0.5})/s_{,inact}])^{-1}$, where I_{max} is the current recorded at -10 mV after the most hyperpolarizing prepulse and $s_{,inact}$ is the slope of the inactivation curve.

Supplementary Tables

Table S1.Summary of clinical characteristics of 14 Brugada syndrome patients with *SCN5A* variants

Age at diagnosis (yrs)	42 ± 12
Gender (M/F)	13/1
Presentation	
Sudden cardiac death	4 (28.5%)
Seizure	2 (14.3%)
Syncope	4 (28.5%)
others	4 (28.5%)
Circumstance	
Sleeping	2 (14%)
Awake	12 (86%)
Documented VF or VT	6 (43%)
Family history of SCD	3 (21.4%)
Spontaneous Type I Brugada ECG	10 (70%)
ICD implantation	7 (50%)

ECG: electrocardiogram; F: female; ICD: implantable cardioveter defibrillator; M: male; SCD: sudden cardiac death; VT: ventricular tachycardia; VF: ventricular fibrillation

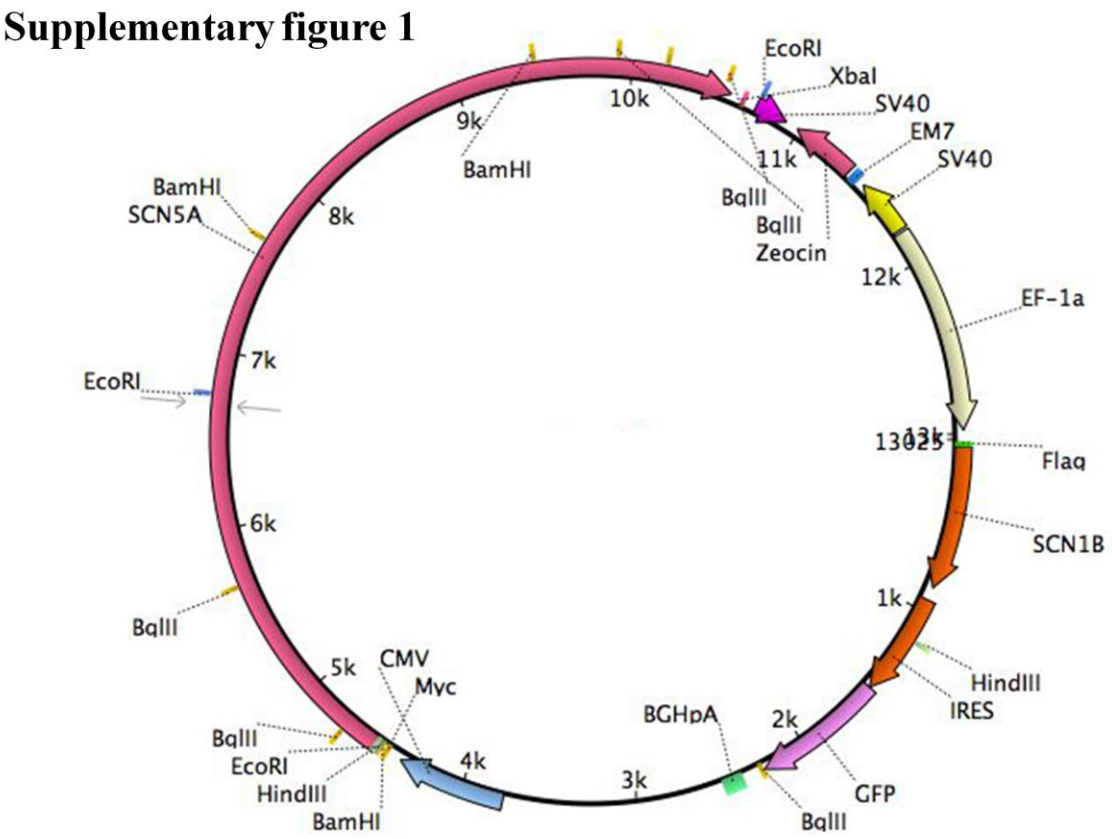
Table S2. Functional predictions and electrophysiological studies in twelve SCN5A mutations validated by in vitro functional studies

Mutation	SIFT	PolyPhen-2	Align-GVGD	EP studies
R18Q	Int	D	Del1	Neutral[6]
R27H	Int	D	Del1	Pathogenic[6]
G35S	T	B	N1	Neutral[6]
L212P	Int	D	Del2	Pathogenic[7]
T353I	Int	D	Del1	Pathogenic[8]
I890T	Int	D	Del1	Pathogenic[9]
R1232W	Int	D	Del1	Neutral[10]
F1344S	Int	D	Del1	Pathogenic[11]
R1432G	Int	D	N1	Pathogenic[12]
R1512W	Int	D	Del1	Pathogenic[12]
T1620M	Int	D	Del1	Pathogenic[10]
S1710L	Int	D	Del1	Pathogenic[13]

B: Benign; D: damaging; Del1: Deleterious 1; Del2: Deleterious 2; EP: electrophysiological; GVGD: Grantham Variation Grantham Deviation; Int: intolerant; N1: Neutral 1; PolyPhen-2: Polymorphism phenotyping-2; SIFT, Sorting Intolerant From Tolerant; T: tolerant

Supplementary Figure

Figure S1. The expression vector construct of human cardiac sodium channel (*SCN5A* and *SCN1B* clone).



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