p.D1690N sodium voltage-gated channel α subunit 5 mutation reduced sodium current density and is associated with Brugada syndrome

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Abstract. Brugada syndrome (BrS) is an inherited primary arrhythmia disorder, leading to sudden cardiac death due to ventricular tachyarrhythmia, but does not exhibit clinical cardiac abnormalities. The sodium voltage-gated channel α subunit 5 (*SCN5A*) gene, which encodes the α subunit of the cardiac sodium channel, Nav1.5, is the most common pathogenic gene, although ≥22 BrS-susceptibility genes have previously been identified. In the present study, a novel genetic variant (p.D1690N) localized in the S5-S6 linker of domain IV of the Nav1.5 channels was identified in a Chinese Han family. Wild-type (WT) and p.D1690N Nav1.5 channels were transiently over-expressed in HEK293 cells and analyzed via the whole-cell patch clamp technique. The p.D1690N mutation significantly reduced the peak sodium current density to 23% of WT (at -20 mV; P<0.01), shifted steady-state activation by 7 mV to increasingly positive potentials (P<0.01). Furthermore, prolonging of the recovery from inactivation was observed in the p.D1690N mutant. No significant change was identified in steady-state inactivation. Thus, the mutant-induced changes contributed to the loss of function of Nav1.5 channels, which indicates that the p.D1690N variant may have a pathogenic role in BrS.

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Abbreviations: I_{Na}, depolarizing inward sodium current; I_{Ca}, depolarizing inward calcium current; I_{Ks}, repolarizing outward slow rectifying potassium current; I_{to}, transient outward potassium current; I_{Kr}, repolarizing outward rapid rectifying potassium current; I_{KATP}, ATP-sensitive inward rectifying potassium current; I_f, funny current; ChIP, channel-interacting protein; BrS, Brugada syndrome; ECG, electrocardiogram; SCD, sudden cardiac death; PVT, polymorphic ventricular tachycardia; VF, ventricular fibrillation; RBBB, right bundle branch block; ER, endoplasmic reticulum

Key words: Brugada syndrome, sodium voltage-gated channel α subunit 5, sudden cardiac death, genetics, patch-clamp technique, channelopathies

Introduction

Brugada syndrome (BrS) is a life-threatening, inherited, primary arrhythmia disorder characterized by ST-segment elevation in the right precordial leads (V1-V3) of the electrocardiogram (ECG), which does not exhibit any clinical cardiac abnormality (1,2). BrS patients may experience syncope and sudden cardiac death (SCD) from episodes of polymorphic ventricular tachycardia (PVT) and ventricular fibrillation (VF), particularly in healthy young males (3). BrS accounts for 4% of all SCD and ≤20% of sudden deaths in patients without obvious structural heart disease (3). BrS is an inherited disease that shows an autosomal dominant pattern with incomplete penetrance and an incidence ranging from ~5 per 10,000 in Western countries to 12 per 10,000 in Southeast Asia (3-5).

To date, although 22 susceptibility genes have been identified in BrS, *SCN5A*, which encodes the α subunit of the major cardiac sodium channel (Nav1.5), is the most common pathogenic gene and is responsible for 11-28% of BrS patients (3,6-8). However, mutations in other genes have rarely been observed in BrS patients and account for the minority (<25%) of BrS genotype-positive cases (8,9). Furthermore, >370 mutations in the *SCN5A* gene have been associated with BrS using a web database, The Gene Connection For The Heart

(http://triad.fsm.it/cardmoc/). Many of these mutations, which have been characterized in cell lines expressing BrS mutant channels revealed a loss-of-function effect on the sodium current by certain mechanisms, such as reduced current density or disrupted biophysical properties (10-12). Thus, the genetic etiology of BrS remains unclear. Identifying novel susceptibility genes will provide clinical benefit for early diagnosis, risk stratification and personalized treatment of BrS.

The present study investigated a Chinese Han patient presenting with BrS carrying a novel heterozygous mutation, p.D1690N [found in the S5-S6 linker of domain IV (DIV) of the Nav1.5 channel] in the *SCN5A* gene. In addition, the functional outcomes of the mutated Nav1.5 channel proteins were examined in HEK293 cells. The results demonstrated that the mutation reduced sodium current density and altered the biophysical sodium channel characteristics.

Materials and methods

Clinical characteristics. A 37-year-old male was admitted to the emergency department of The First Affiliated Hospital of Xiamen University (Xiamen, China) in September 2009, due to sudden syncope from an episode of VF (Fig. 1A). A subsequent 12-lead ECG at rest was consistent with a type-1 Brugada pattern, defined as a prominent coved-type ST-segment elevation, displaying ST-segment elevation >2 mm at its peak followed by a negative T wave (3) and incomplete right bundle branch block (RBBB; Fig. 1B). The BrS patient demonstrated no evidence of structural heart disease by exercise stress test, electrophysiological study and echocardiography. Subsequently, the family of the patient (four males and three females; mean age, 38.9±17.6 years) underwent physical examination, 12-lead ECG, 24-h Holter ECG monitoring (Nihon Kohden, Tokyo, Japan), echocardiography and genetic testing. As ajmaline, flecainide and procainamide, recommended by the consensus report (13), are unobtainable in China, propafenone (Shanghai Xinyi Jinzhu Pharmaceutical Co., Ltd., Shanghai, China) was administered, which has been demonstrated to reveal BrS. Propafenone challenges were performed and evaluated on two of the family members [the sister (II.3) and nephew (III.2) of the proband] as previously described (13). The subjects provided written informed consent and, following a fasting period (~10 h overnight), propafenone (1 mg/kg body weight; 10 mg/min) was intravenously administered while the patient was continuously monitored by 12-lead ECG and blood pressure. After 20 min, if the reaction was negative (i.e. no change in ECG pattern compared with the baseline, including absence of ST segment elevation and QRS duration prolongation), an additional 0.5 mg/kg propafenone was injected for 2.5 min. Propafenone infusion was terminated when the diagnostic type I Brugada ECG pattern or ventricular arrhythmias were apparent. After termination of propafenone administration, monitoring was continued for a minimum of 24 h or until the ECG normalized. The propafenone challenge was performed and evaluated on two family members, II.3 and III.2, who were carriers of the *SCN5A* mutation.

Genetic analysis. The current study conforms with the principles outlined in the Helsinki Declaration and was approved by the Medical Ethical Committee of The First Affiliated Hospital of Xiamen University. All subjects provided written informed consent following counseling. Genomic DNA was isolated from leukocyte nuclei using a TIANamp Blood DNA kit (Tiangen Biotech Co., Ltd., Beijing, China), and the cardiac sodium channel gene, *SCN5A* (transcript, NM_198056.2) was directly sequenced following polymerase chain reaction (PCR) amplification, with the use of an ABI PRISM 3730xl DNA sequencer (Thermo Fisher Scientific, Inc., Waltham, MA, USA) performed by Tsingke Biological Technology Co., Ltd. (Beijing, China), as previously described (12). All DNA-identified variants were compared with a control group of 150 healthy and unrelated Chinese Han individuals (300 alleles) after obtaining written informed consent.

Site-directed mutagenesis and heterologous expression. The p.D1690N mutation was introduced into pcDNA3.1-hH1 using a PCR-based mutagenesis method, as previously described (12). The mutated plasmids were sequenced (Tsingke Biological Technology Co., Ltd.) to ensure the presence of the p.D1690N mutation and the absence of spurious mutations.

The human embryonic kidney 293 cell line, HEK293 was cultured in an incubator in Gibco Dulbecco's modified Eagle's medium (Thermo Fisher Scientific, Inc.) supplemented with Gibco 10% fetal bovine serum (Thermo Fisher Scientific, Inc.), 4 mmol/l glutamine (Invitrogen; Thermo Fisher Scientific, Inc.), 100 IU/ml penicillin (Amresco LLC, Solon, OH, USA) and 100 μg/ml streptomycin (Amresco LLC) at 37˚C in a humidified atmosphere of 5% CO_2. Prior to over-expression, the cells were seeded in 6-well plates and, upon reaching 80% confluence, were co-transfected with 0.8 μg pcDNA3.1-hH1 or p.D1690N mutant and 0.8 μg pIRES2-DsReD-sodium voltage-gated channel β subunit 1 (*SCN1B*; which served as a reporter gene) with Invitrogen Lipofectamine 2000 (Thermo Fisher Scientific, Inc.), according to the manufacturer's instructions and as previously reported (12).

Electrophysiological analysis. Twenty-four to 48 h after transfection, the sodium current (I_{Na}) was recorded in cells displaying red fluorescence at room temperature (23-25˚C) under whole cell patch-clamp technique, as previously described (12). Briefly, the cells were continuously superfused with a bath solution containing 70 mM NaCl, 80 mM CsCl, 5.4 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES and 10 mM glucose (pH adjusted to 7.3 using CsOH). The patch pipette (tip resistance, 2-3 MΩ) was filled with intracellular medium containing 20 mM NaCl, 130 mM CsCl, 10 mM HEPES and 10 mM EGTA (pH adjusted to 7.2 with CsOH). All the above-mentioned items were purchased from Sigma-Aldrich (St. Louis, MO, USA). The sodium current was recorded using a MultiClamp™ 700B amplifier (Molecular Devices, LLC, Sunnyvale, CA, USA) and was converted to digital data using a Digidata® 1440A A/D converter (5 kHz filtering; Molecular Devices, LLC).

Statistical analysis. Continuous results are expressed as means ± standard error of the mean. Currents were analyzed using Clampfit 10.1 software (Molecular Devices, LLC, Sunnyvale, CA, USA) and Origin Pro 8.5 (OriginLab Corporation,Northampton, MA, USA). Statistical comparisons

Table I. Susceptibility genes for BrS.

Type of BrS	Gene	Ion-channel component	Functional effect	Carriers among BrS patients (%)
BrS 1	*SCN5A*	α subunit I_{Na}	Loss of function	11-28
BrS 2	*GPD1-L*	I_{Na} ChIP	Loss of function	<1
BrS 3	*CACNA1c*	α subunit I_{Ca}	Loss of function	3-4
BrS 4	*CACNB2b*	β subunit I_{Na}	Loss of function	2-3
BrS 5	*SCN1B*	β subunit I_{Na}	Loss of function	<1
BrS 6	*KCNE3*	β subunit I_{Ks}/I_{to}	Gain of function	<1
BrS 7	*SCN3B*	β subunit I_{Na}	Loss of function	<1
BrS 8	*KCNH2*	α subunit I_{Kr}	Gain of function	<1
BrS 9	*KCNJ8*	α subunit I_{KATP}	Gain of function	<1
BrS 10	*CACNA2D1*	α2δ subunit I_{Ca}	Loss of function	<1
BrS 11	*RANGRF*	I_{Na} ChIP	Loss of function	<1
BrS 12	*KCNE5*	β subunit I_{to}	Gain of function	<1
BrS 13	*KCND3*	α subunit I_{to}	Gain of function	<1
BrS 14	*HCN4*	I_f	Loss of function	<1
BrS 15	*SLMAP*	I_{Na} ChIP	Loss of function	<1
BrS 16	*TRMP4*	α subunit	Loss of function	6
BrS 17	*SCN2B*	β subunit I_{Na}	Loss of function	<1
BrS 18	*FGF12*	I_{Na} ChIP	Loss of function	<1
BrS 19	*ABCC9*	I_{KATP} CHIP	Gain of function	1
BrS 20	*PKP2*	I_{Na} ChIP	Loss of function	2.5
BrS 21	*SEMA3A*	I_{to} CHIP	Gain of function	1
BrS 22	*SCN10A*	α subunit I_{Na}	Loss of function	16.7

I_{Na}, depolarizing inward sodium current; I_{Ca}, depolarizing inward calcium current; I_{Ks}, repolarizing outward slow rectifying potassium current; I_{to}, transient outward potassium current; I_{Kr}, repolarizing outward rapid rectifying potassium current; I_{KATP}, ATP-sensitive inward rectifying potassium current; I_f, funny current; ChIP, channel-interacting protein; BrS, Brugada syndrome.

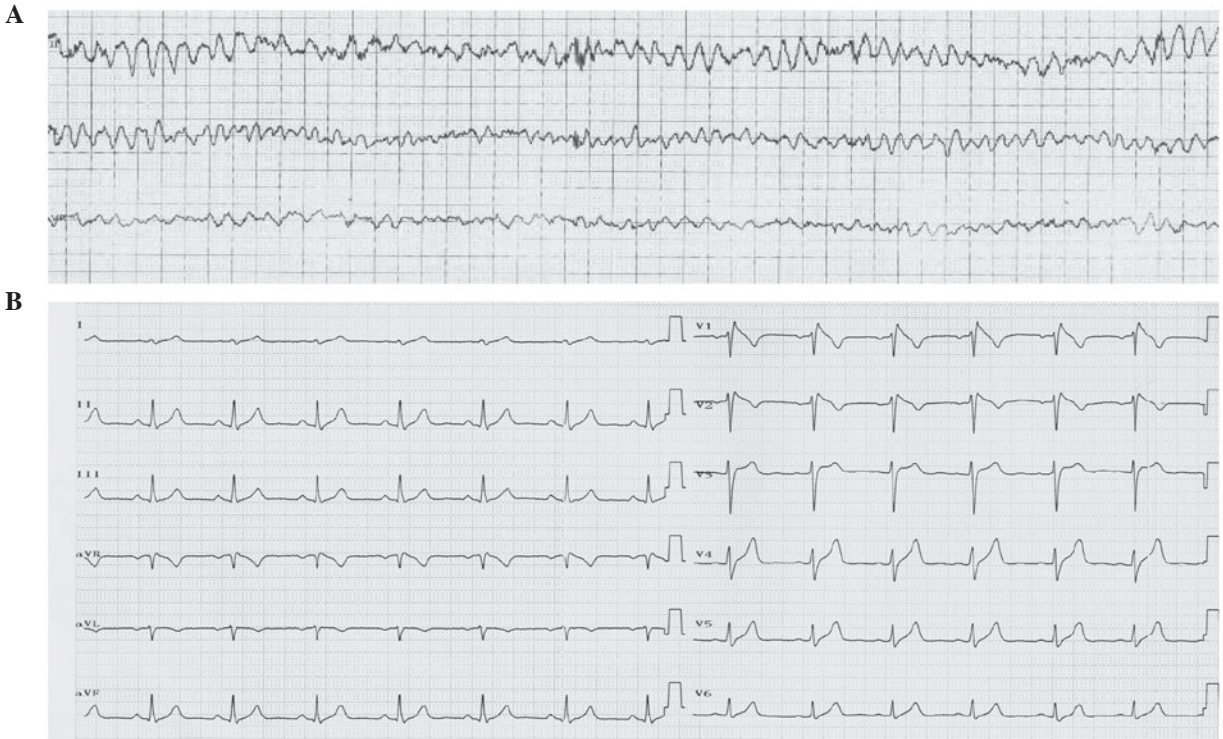

Figure 1. Clinical manifestations of the proband. (A) ECG recording of the proband demonstrating ventricular fibrillation. (B) Representative 12-lead surface ECG recording of the proband showing prominent coved-type ST-segment elevation, following a negative T wave in V1-V2 and incomplete right bundle branch block, which is typically observed in type-1 Brugada syndrome (paper speed, 25 mm/sec). ECG, electrocardiogram.

between the WT and mutation groups were evaluated using the two-tailed Student's *t*-test and P<0.05 was considered to indicate a statistically significant difference.

Results

Clinical studies. The present study included seven members of a Chinese Han family with BrS and a history of SCD (the genetic background is presented in Fig. 2A). The proband, a 37-year-old male, was admitted to the emergency department of The First Affiliated Hospital of Xiamen University due to sudden syncopes at night. An ECG demonstrated VF (Fig. 1A), which was treated by electric defibrillation. The clinical history revealed that the patient's father (I.1) succumbed suddenly at the age 53 years. The patient experienced PVT three times in hospital, each of which were successfully treated by electric defibrillation.

At rest, the ECG demonstrated sinus rhythm and was compatible with the type-1 Brugada pattern (Fig. 1B), showing ST-elevation in V1-V3 followed by negative T wave and RBBB. No structural heart diseases or valvular disease were observed by echocardiography. The proband refused an implantable cardioverter defibrillator, as a result of financial constraints, and succumbed two months later due to SCD. Following complete cardiologic examination, the majority of the patient's relatives exhibited normal ECG patterns and echocardiograms, although the patient's nephew (III.2) exhibited a BrS diagnostic ECG and the patient's sister (II.3) exhibited a negative reaction following propafenone challenge.

Molecular genetic findings. By sequencing *SCN5A*, a novel missense mutation, c.5262G > A, which was identified in three family members (II.2, II.3 and III.2), is predicted to replace aspartic acid (D) with asparagine (N) at position 1690 (D1690N) in the DIV S5-S6 linker of the Nav1.5 channels (Fig. 2). The variant was not detected in 150 ethnically matched, healthy volunteers (300 alleles) and The Human Gene Mutation Database (14) ruled out the polymorphism.

Additionally, the common single nucleotide polymorphism, c.1673A > G, which produces the p.H558R polymorphism, was not detected in the family members.

Electrophysiology of p.D1690N Nav1.5 channels. To characterize the electrophysiological consequences of the p.D1690N mutation on Nav1.5 channel activity, the biophysical properties of p.D1690N mutant with WT recombinant human sodium channels, combined with the hβ1 subunit in HEK293 cell lines, were compared using the whole-cell patch-clamp technique. As shown in Fig. 3A and B, the mutation significantly reduced the peak current densities of Nav1.5 [-20-mV current density: WT (n=31), -336.7±26.9 pA/pF vs. mutant (n=34), -93.3±9.0 pA/pF; P<0.01]. Therefore, it was hypothesized that the position, D1690 may be key in the trafficking and/or stability of the channel.

As shown in Fig. 3C, the steady-state activation and inactivation curves of the WT and mutant were obtained by plotting the normalized peak current as the corresponding membrane voltage. The midpoint of activation ($V_1/_2$ act) of the p.D1690N mutant was markedly shifted by 7 mV to more positive potentials when compared with the WT channels [$V_1/_2$ act: -38.2±1.7 mV, (n=14) vs. -31.2±1.6 mV (n=12) for WT and mutant channels, respectively; P<0.01, Fig. 3C]. By contrast, no significant differences were observed in the voltage dependence of steady-state inactivation [$V_1/_2$ inact: -86.0±1.5 mV (n=15) vs. -82.2±1.6 mV (n=13) for WT and mutant channels, respectively; Fig. 3C]. These results revealed that the mutation accelerated activation kinetics. In addition, the recovery constant from fast inactivation for mutant channels was significantly delayed when compared with WT channels [τf=3.9±0.3 msec, τs=57.1±11.0 msec (n=14) vs. τf=5.9±0.4 msec (P<0.01 vs. WT), τs=73.9±9.7 msec (n=15) for WT and mutant channels, respectively; Fig. 3D].

Discussion

In the present study, the functional consequences of a novel missense mutation, p.D1690N, which was identified in a patient with repeated episodes of VF, and whose ECG presents the typical features of BrS under basal conditions, was investigated. The electrophysiological analysis revealed that the p.D1690N mutation results in a significant loss of function in the sodium channel, which was predominantly attributable to the reduction in current density and abnormal kinetic properties. Numerous studies indicate that *SCN5A* mutations associated with BrS result in loss of function of sodium channels (10,15-17). The present results indicate that the BrS in this particular family was attributed to the novel missense mutation, p.D1690N in the Nav1.5 channels.

BrS is an inherited channelopathy that is characterized by ST-segment elevation in the right precordial leads (V1-V3) of the electrocardiogram, but that does not demonstrate any cardiac structural disease. To date, 22 BrS-susceptibility genes have been identified (Table I), primarily via candidate gene approaches (3,6-8). These genes encode subunits of sodium channels and their interacting proteins [*SCN5A*, sodium voltage-gated channel α subunit 10 (*SCN10A*), *SCN1B*, sodium voltage-gated channel β subunit 2, sodium voltage-gated channel β subunit 3, glycerol-3-phosphate dehydrogenase 1-like, RAN guanine nucleotide release factor, fibroblast growth factor 12, plakophilin 2, sarcolemma associated protein], potassium channels and their interacting proteins (potassium voltage-gated channel subfamily H member 2, potassium voltage-gated channel subfamily E regulatory subunit 3, potassium voltage-gated channel subfamily E regulatory subunit 5, potassium voltage-gated channel subfamily D member 3, potassium voltage-gated channel subfamily J member 8, semaphorin 3A, ATP binding cassette subfamily C member 9), L-type calcium channels [calcium voltage-gated channel subunit α1 C (*CACNA1C*), calcium voltage-gated channel auxiliary subunit β and calcium voltage-gated channel auxiliary subunit α2δ 1] and other channels (transient receptor potential cation channel subfamily M member 4 and hyperpolarization activated cyclic nucleotide gated potassium channel 4) (6,18,19). The *SCN5A* mutations account for 11-28% of all BrS patients (8) and the prevalence of the *CACNA1C* mutation ranges from 2 to 12% in the literature (20,21). Recently, Hu *et al* (9) identified that mutations in the *SCN10A* gene, encoding the Nav1.8 channel located adjacent to *SCN5A* on chromosome 3p21-22, contribute to 16.7% cases of BrS. However, another study revealed that although *SCN10A* variants demonstrated a loss-of-function

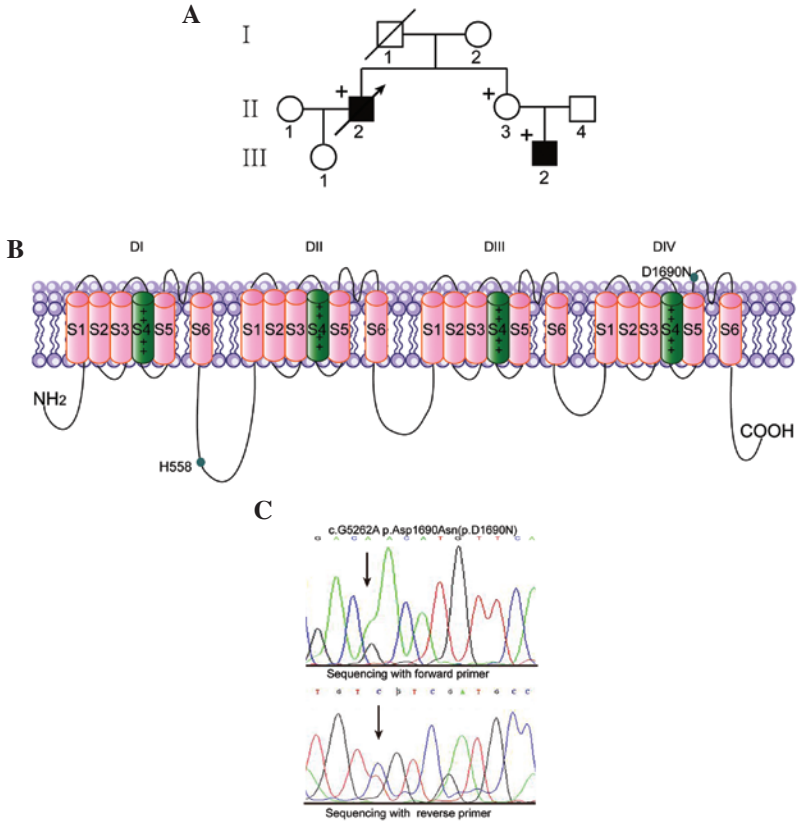

Figure 2. Genetic analysis and background of the proband and family members. (A) The genetic background and mutation status of the Chinese Han family: Males, squares; females, circles; BrS phenotype, filled symbols; variation carrier for SCN5A c.5262G > A, + symbol; proband, arrow. (B) Location of the p.D1690N mutation and the polymorphism in the predicted topological diagram of the Nav1.5 channel. (C) Polymerase chain reaction-based sequence of *SCN5A* exon 28 demonstrating a heterozygous nucleic acid substitution (c.5262G > A) in the proband, the sister (II.3) and the nephew (III.2), resulting in a single amino acid substitution, p.Asp1690Asn (p.D1690N). SCN5A, sodium voltage-gated channel α subunit 5; D, domain.

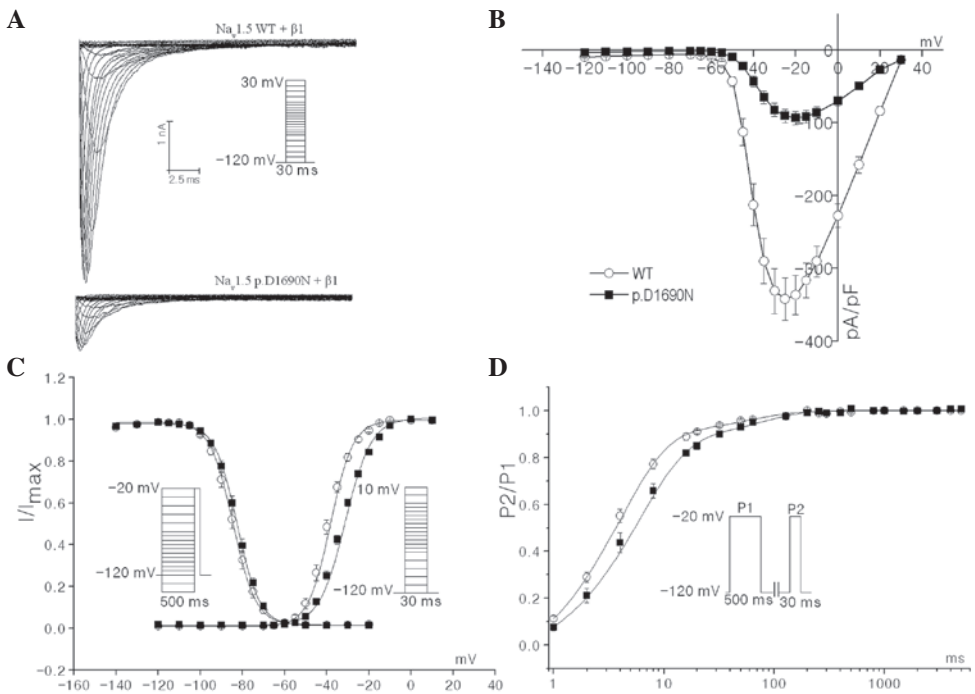

Figure 3. p.D1690N mutation decreases sodium current density and alters the electrophysiological kinetics of Nav1.5 channels. (A) Representative current traces of cells transfected with WT and p.D1690N channels. (B) The current density-voltage association for WT (open circles) and p.D1690N (filled square) channels. (C) Steady-state activation and inactivation curves of WT (open circles) and p.D1690N (filled square) channels obtained by applying the protocol in the inset. (D) Time constants of recovery from fast inactivation was investigated using a twin-pulse protocol (inset); τf=3.9±0.3 msec, τs=57.1±11.0 msec (n=14). vs. τf=5.9±0.4 msec (P<0.01 vs. WT), τs=73.9±9.7 msec (n=15) for WT and mutant channels, respectively. Data are presented as means ± standard error of the mean. WT, wild-type.

during *in vitro* electrophysiological experiments, those variants are not significantly associated with BrS (22). In addition, novel burden testing revealed that *SCN5A* alone is responsible for a significant proportion of BrS cases, and other genes, including *SCN10A* and *CACNA1C*, do not demonstrate enrichment in rare coding variation (6). Thus, this should be noted to avoid diagnosing false-positive cases of causality during genetic counseling, as rare coding variations in BrS-susceptibility genes are also common in healthy individuals.

Genetic screening revealed that two family members of the proband (II.3 and III.2) were carrying the mutation. BrS is a genetically and clinically heterogeneous disease and presents in individuals aged several-months to 80-years-old, although more typically at the average age of 40 years (13). When the manifestations of BrS are not shown during ECG diagnostics, drug challenge is recommended to reveal asymptomatic first-degree relatives of BrS patients (13). For the nephew of the proband (III.2; age, 19 years), BrS was definitively diagnosed following a positive propafenone challenge and in conjunction with type 1 pattern ECGs of the family members. The ECG signature of BrS is dynamic and can be undetected for a period of time. Numerous factors, such as sex hormones, age and fever, have been proposed as being potentially responsible for the phenomenon of BrS (23,24). Thus, repeated drug challenges or 12-lead, 24-h Holter monitoring are highly recommended for asymptomatic children with a family history of BrS (25,26).

The p.D1690N mutation causes a negative charge to a neutral amino acid substitution in the pore region between the DIVS5 and DIVS6 transmembrane segments that significantly affects channel expression and gating kinetics. A central ion-conducting pore of Nav1.5 channels is formed by the S5 and S6 segments and their linker extracellular loops, determining ion selectivity. Núñez *et al* (15) identified two p.D1690N and p.G1748D compound heterozygous mutations in the Nav1.5 channel in a Caucasian family with a history of BrS. Notably, p.D1690N completely restores the gating defects presented by p.G1748D channels; however, has little impact on its trafficking. However, the p.D1690N mutation alone has not been identified in BrS patients. In the present study, a missense variant, p.D1690N was identified in the Nav1.5 channel in three of the proband's family members (II.2, II.3 and III.2). During comparisons of the electrophysiological characteristics of the WT and p.D1690N mutant in HEK293 cells, it was identified that the key alteration of the p.D1690N mutation was the dramatic reduction in I_{Na} density (~72%) predominantly attributable to a significant impairment of channel trafficking toward the membrane. In addition, the p.D1690N mutant delayed the time of channel recovery from inactivation induced by depolarization. These changes caused by the p.D1690N mutation may reduce the activity of the Nav1.5 channel and contribute to BrS.

There have been various studies investigating the underlying mechanisms of decreasing current density by missense mutations. One mechanism is that the mutant channels fail to properly traffic to the cytomembrane and are detained in the endoplasmic reticulum (ER) by a quality control system, such as R1432G (11). The quality control system in the ER initially arrested and ultimately degraded the mutation-induced incorrectly folded proteins (11). A second mechanism is that certain mutations, localized in the ion-conducting pore, may block Na^+ permeation, inducing a significant reduction in the sodium current through the channels (27). Consistent with previous results, the present study identified that the mechanism underlying the reduction in sodium current density is the trafficking disorders due to the p.D1690N mutation.

A previous report demonstrated that pharmacological interventions (such as administration of mexiletine) significantly increased current density in a G1743R mutant by rescuing its expression levels in the plasma membrane (28). Although pharmacological interventions provide potential clinical benefit to BrS patients carrying the mutation, whether the p.D1690N mutant channel may be rescued by mexiletine administration requires further investigation. In addition, various studies revealed that the p.H558R polymorphism restores the biophysical defects caused by numerous loss-of-function *SCN5A* mutations underlying sudden infant death syndrome, cardiac conduction disease and BrS (29-31). In the *SCN5A* mutations associated with BrS, p.H558R increases the current density generated by p.R282H (32), p.D1690N (15), p.S216L (33) and p.K317N (34), predominantly by mitigating their impaired trafficking. Thus, the absence of p.H558R in this family fails to restore the trafficking defect in D1690N in the *SCN5A* gene encoding cardiac Na^+ channels (such as Nav1.5).

In conclusion, a novel heterozygous human mutation (D1690N), in the DIV S5-S6 linker of the Nav1.5 channel, was identified in a Chinese Han patient with BrS and the patient's family. The results indicate that the marked reduction of sodium current density in the p.D1690N mutant is attributable to trafficking disorders of the Nav1.5 channel proteins to the sarcolemma. The decreased sodium current density and abnormal activities caused by the p.D1690N mutation are consistent with the hypothesis that a loss of function of Nav1.5 contributes to the pathogenesis of BrS. Whether the characterizations of the p.D1690N mutant exist *in vivo* and the precise mechanisms require further research. However, the present study strengthens the understanding of the association between the structure and function of the Nav1.5 channel, and provides potential personalized therapeutic approaches for inherited cardiac arrhythmia.

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