Characterization of a novel SCN5A mutation associated with Brugada syndrome reveals involvement of DIIIS4–S5 linker in slow inactivation

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Abstract

Objective: Mutations in SCN5A, the gene encoding the α-subunit of the cardiac sodium channel (Na,1.5), have been associated with various inherited arrhythmia syndromes, including Brugada syndrome (BrS). Here, we report the functional consequences of a novel missense SCN5A mutation, G1319V, identified in a BrS patient. The G1319V mutation is located in the loop connecting transmembrane segments 4 and 5 in domain III (DIIIS4–S5), a region so far considered to be exclusively involved in fast inactivation.

Methods: Whole-cell mutant (G1319V) and wild-type (WT) sodium currents (INa) were studied in the Human Embryonic Kidney cell line (HEK-293) transfected with Nav1.5 α-subunit cDNA (WT or mutant) together with hβ1-subunit cDNA, using the patch-clamp technique.

Results: Maximal peak INa and persistent sodium current were similar in WT and channel G1319V channels. The G1319V mutation shifted the potential of half-maximal (V1/2) activation towards more positive potentials (+3.7 mV), thereby increasing the degree of depolarization required for activation. The V1/2 of inactivation of G1319V channels was shifted by −6.0 mV compared to WT, resulting in a reduced channel availability. The change in the steady-state inactivation was completely due to a negative shift (−6.8 mV) of the voltage-dependence of slow inactivation, while the voltage-dependence of fast inactivation was unaffected. The fast component of recovery from inactivation of G1319V channels was slowed down. Finally, the G1319V mutation caused a two-fold increase in the propensity of the channels to enter the slow inactivated state. Reduction in INa peak amplitude on repetitive depolarizations at short interpulse intervals (40 ms) was significantly more pronounced in G1319V compared to WT. Accordingly, carriers of the G1319V mutation showed marked QRS widening upon increases in heart rate during exercise testing, pointing to enhancement of slow inactivation.

Conclusions: We identified the DIIIS4–S5 linker as a new region involved in slow inactivation of Na,1.5. The biophysical alterations of the G1319V mutation all contribute to a reduction in INa, in line with the proposed mechanism underlying BrS.

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Keywords: Arrhythmia (mechanisms); ECG; Membrane currents; Na channel; Sudden death

1. Introduction

The cardiac sodium channel (Na,1.5) is critically involved in cardiac excitability and impulse propagation, because it initiates the action potential (AP) of the specialized conduction system and working myocytes. Mutations in SCN5A, the gene which encodes the pore-forming α-subunit of Na,1.5, are associated with various
inherited arrhythmia syndromes. Brugada syndrome (BrS), characterized by ST-segment elevation in leads V1–V3, and progressive cardiac conduction disease (PCCD), manifesting as a progressive impairment in cardiac conduction (e.g., widening of the QRS complex), are both linked to SCN5A mutations which cause reduced sodium current ($I_{\text{Na}}$) [1,2]. Accordingly, SCN5A-related BrS and PCCD may exhibit substantial phenotypical overlap, i.e., BrS is often associated with marked conduction slowing [3]. Thus, in addition to the current hypothesis [4], recent studies on how reduced $I_{\text{Na}}$ can cause BrS, revolve around conduction slowing in the right ventricle (RV) [5–7]. In contrast to the loss-of-function mutations described in BrS and PCCD, a gain-of-function of the Na$_\alpha$1.5 channel is observed in the long QT syndrome type 3 (LQT3), manifesting on the ECG as a prolonged QT interval [8].

These differences in phenotypes are explained by changes in channel expression and/or gating properties of Na$_\alpha$1.5. In particular, alterations in the fast and slow inactivation processes play a prominent role [8–10]. Whereas fast inactivation occurs in few milliseconds, sodium channels enter a ‘slow’ inactivated state upon prolonged depolarization, characterized by a lifetime ranging from hundreds of milliseconds to many seconds. An ‘intermediate’ kinetic component of inactivation develops in Na$_\alpha$1.5 with a time course similar to the duration of the cardiac AP (hundreds of milliseconds) [9,10]. We will refer to this component with the term “slow inactivation”. Fast and slow inactivations are kinetically and structurally different processes. Mutations of the structure necessary for fast inactivation, the IFM motif (isoleucine–phenylalanine–methionine) in the DIII–DIV linker, completely remove fast inactivation [11], but leave slow inactivation intact [12].

Clearly, distinguishing the different clinical phenotypes resulting from SCN5A mutations is crucial for optimal patient management. At the same time, defining the topology of various Na$_\alpha$1.5 gating properties may aid the development of new Na$_\alpha$1.5 targeting antiarrhythmic drugs. Here, we describe the functional properties of an SCN5A missense mutation, G1319V, identified in individuals with BrS. This mutation is located in DIII3–S5, a region hitherto considered to be exclusively involved in fast inactivation [13–15]. Accordingly, previously described mutations located here result in the LQT3 phenotype. Yet, heterologous expression in HEK-293 cells of G1319V channels revealed enhanced slow inactivation with only minor modifications in fast inactivation. Thus, this study identifies a new region involved in slow inactivation.

2. Methods

2.1. Patients

The study was performed according to a protocol approved by the local ethics committees. Informed consent was obtained from all patients.

2.2. Genetic screening

Genomic DNA was extracted from peripheral blood leucocytes by standard procedures. Genetic analysis of SCN5A was done as described previously [16]. Our study and all experiments conform to the Declaration of Helsinki.

2.3. Site-directed mutagenesis

Mutant Na$_\alpha$1.5 cDNA was created by site-directed mutagenesis performed on SCN5A cDNA cloned in the pSP64T-hH1 plasmid, as previously described [16]. The G1319V cDNA was then subcloned into the pCGI vector for bicistronic expression of the channel protein and GFP reporter in a Human Embryonic Kidney cell line (HEK-293).

2.4. Heterologous expression of mutant and wild-type sodium channels

To express mutant (G1319V) and wild-type (WT) Na$_\alpha$1.5, HEK-293 cells were cotransfected with 0.5 µg Na$_\alpha$1.5 α-subunit cDNA (WT or G1319V) and 0.5 µg hβ1γ-subunit cDNA with the use of Lipofectamine (Gibco BRL, Life Technologies). Transfected HEK-293 cells were cultured in minimum essential medium (Earles salts and l-glutamine) supplemented with nonessential amino acid solution, 10% fetal bovine serum, 100 IU/mL penicillin, and 100 µg/mL streptomycin in a 5% CO$_2$ incubator at 37 °C for 1–2 days. Cells exhibiting green fluorescence were selected for electrophysiological experiments.

2.5. Electrophysiological measurements and data analysis

Sodium currents ($I_{\text{Na}}$) were recorded in the whole-cell configuration of the patch-clamp technique. Experiments were conducted at room temperature (20 °C) with the use of an Axopatch 200B amplifier (Axon Instruments). Series resistance was compensated by ≥80% and patch electrodes had a tip resistance of 2–3 MΩ. Currents were filtered at 5 kHz and digitized at 20 kHz. Voltage control, data acquisition, and analysis were performed with custom-made software. The presence of a persistent inward sodium current ($I_{\text{Na,p}}$), voltage-dependence of activation, steady-state inactivation, recovery from inactivation, development of slow inactivation, and voltage-dependence of slow and fast inactivation were determined using voltage-clamp protocols described in the relevant figures. In all protocols, holding potential of −120 mV and a cycle time of 5 s were used.

Voltage-dependence of activation and inactivation curves was fitted with a Boltzmann function ($y = \frac{y_0 + A_f \left(1 - \exp\left[-t / \tau_f\right]\right)}{1 + \exp\left(V - V_{1/2}\right) / k} + A_s \left(1 - \exp\left[-t / \tau_s\right]\right)$, where $V_{1/2}$ is the half-maximal voltage of (in)activation and $k$ the slope factor. Recovery from inactivation was analyzed by fitting the data with a two-exponential function ($y = y_0 + A_f \left(1 - \exp\left[-t / \tau_f\right]\right) + A_s \left(1 - \exp\left[-t / \tau_s\right]\right)$, where $A_f$ and $A_s$ indicate the fractions of the fast and the slow components of recovery from inactivation, and $\tau_f$ and $\tau_s$ the
time constants for recovery from fast and slow inactivation, respectively. $I_{Na}$ decay was fitted with a two-exponential function 

$$y = y_0 + A_f(1 - \exp[-t/\tau_f]) + A_s(1 - \exp[-t/\tau_s]),$$

where $A_f$ and $A_s$ represent the amplitudes of the fast and the slow inactivating components, and $\tau_f$ and $\tau_s$ the fast and slow time constants of inactivation, respectively. Development of slow inactivation was fitted with a single exponential function 

$$y = A + A_0 \exp(-t/\tau),$$

where $\tau$ is the time constant for the development of slow inactivation, and $A$ the fraction of channels that enter the slow inactivated state at the steady-state.

The bath solution contained (mmol/L): NaCl 140, KCl 4.7, CaCl$_2$ 1.8, MgCl$_2$ 2.0, NaHCO$_3$ 4.3, Na$_2$HPO$_4$ 1.4, glucose 11.0, HEPES 16.8, pH adjusted to 7.4 (NaOH). Pipettes were filled with (mmol/L): CsF 110, CsCl 1.0, EGTA 11, NaF 10, CaCl$_2$ 1.0, MgCl$_2$ 1.0, Na$_2$ATP 2.0, HEPES 10, pH adjusted to 7.3 (CsOH).

2.6. Sequence alignments and protein modelling

Residues 1262–1372 of SCN5A were aligned against the corresponding residues from the S3–S5 region of the human cyclic nucleotide gated channel alpha 1 (CNGA1) and the human potassium voltage-gated channel (KCNA2). Initial alignments were generated based on those of Anselmi et al. [17] followed by realignment using ClustalX [18]. The alignment was subsequently manually readjusted to provide the best fit to the template (PDB 2a79) before modelling submission. Models were Whatif97 checked.

2.7. Statistical analysis

Results are expressed as mean±SEM. Comparisons were made using unpaired Student’s $t$-test or Mann–Whitney test.

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Fig. 1. ECGs (25 mm/s, 10 mm/mV) of patient 1 (A), patient 2 at baseline (B) and upon ajmaline testing (C), and patient 3 at baseline (D) and during exercise testing (E). V1IC3 and V2IC3, ECG leads positioned over 3rd intercostal space cranial from V1 and V2, respectively.
when data were not normally distributed, and two-way ANOVA with repetitive measurements followed by a Holm–Sidak test for post-hoc analysis, where appropriate. $p < 0.05$ indicates statistical significance.

3. Results

3.1. Clinical and genetic studies

The proband, a 42-year-old Caucasian man (patient 1), sustained ventricular fibrillation in the passenger seat of a car. He had no medical history, prior syncope, or family history of sudden death. Cardiologic investigation revealed no structural heart disease (negative cardiac enzymes, unremarkable echocardiography, further investigations declined). The ECG showed conduction slowing: QRS complexes were fractionated, widened (150 ms), and had left axis deviation, with first degree AV block (PQ 0.24 s). Moreover, ST segments in right precordial leads were elevated, compatible with BrS (Fig. 1A). No signs of LQT syndrome were present, and QT/QTc were normal (360/377). Sequence analysis of SCN5A revealed a g>t substitution at position 3956 (numbering based on cDNA RefSeq: NM_198056), leading to the replacement of glycine by valine (ggc>gtc) at residue 1319 in DIIIS4–S5 of Na$^+$,1.5 (G1319V, Fig. 2A). Glycine at that position is highly conserved.

![Diagram of SCN5A mutation](https://example.com/diagram.png)

Fig. 2. (A) Schematic drawing of the cardiac voltage-gated Na$^+$ channel α-subunit (Na$^+$,1.5) showing the position of the G1319V mutation in the linker between segments 4 and 5 in domain III (DIIIS4–S5). In the enlargement of A all known missense mutations located in DIIIS4–S5 are indicated. (B) Diagram of amino acid composition of DIIIS4–S5 of different voltage-gated sodium channel isoforms and species showing that the amino acid glycine at the 1319 position is highly conserved.
conserved amongst different species (Fig. 2B), and this mutation was not detected in 400 alleles of normal control individuals with the same ethnic profile.

During implantation of a cardioverter-defibrillator (ICD), intracardiac electrogram amplitudes in the RV were low and defibrillation thresholds high. At ICD replacement 6 years later, defibrillation thresholds were also high. During a 6-year follow-up, he received 2 appropriate ICD shocks for rapid ventricular tachycardia (average cycle length 190 ms). Family screening revealed that his mother (cardiological investigation declined) and his 41-year-old brother (patient 2) also carried the G1319V mutation. The brother’s cardiac MRI was normal, but his baseline ECG revealed QRS widening (120 ms, Fig. 1B), and the signature coved-type ST-segment changes of BrS were induced by ajmaline drug testing (Fig. 1C). Exercise testing evoked further QRS widening upon increases in heart rate (baseline: QRS 120 ms at 83 beats per minute [bpm]; peak exercise: QRS 130 ms at 179 bpm). QRS widening at fast heart rates during exercise testing was also observed in another, unrelated, BrS patient (patient 3), a 34-year-old woman, who carried the G1319V mutation. The brother’s cardiac disease, ajmaline testing elicited a typical BrS response.

3.2. Electrophysiological characterization of the G1319V sodium channel

We studied the electrophysiological properties of WT and G1319V channels after heterologous expression in HEK-293 cells. Despite inherent limitations of this system [20], this is a well accepted method to assess the functional consequences of a mutation. Results are summarized in Table 1. WT and G1319V I_{NaL} were evoked by increasing depolarizing steps from a holding potential of −120 mV (Fig. 3A). As shown by the average current–voltage relations (Fig. 3C), maximal peak current amplitudes were not significantly different between both groups (WT: I_{peak,max} = 5.8 ± 0.7 nA, n = 15; G1319V: I_{peak.max} = 5.8 ± 0.6 nA, n = 20), indicating that the number of functional channels is similar for WT and mutant.

I_{NaL} was analyzed as a 30 μmol/L tetrodotoxin (TTX)-sensitive current, using a descending voltage ramp protocol (Fig. 3D). Representative examples of traces obtained from WT (n = 6) and G1319V channels (n = 6), by subtraction of the current before and after application of TTX, are shown in Fig. 3B. Over the range of tested membrane potentials, G1319V channels had no detectable I_{NaL} (Fig. 3D). The normalized conductance–voltage relation (Fig. 4A), showed a small depolarizing shift (+3.7 mV, p = 0.04) in voltage-dependence of activation for G1319V channels compared to WT channels with no change in slopes. Thus, the degree of depolarization required for activation was increased in G1319V channels.

To analyze the inactivation kinetics, we fitted the whole-cell current decay at various potentials with a two-exponential function. The slow time constant of current decay, τ_s, was slightly prolonged in G1319V compared to WT, but only significantly between −50 and −40 mV. The amplitudes of the fast and slow inactivating components of the current decay (A_f and A_s) were comparable for WT and G1319V channels at all voltages (not shown).

Investigation of the voltage-dependence of steady-state inactivation (Fig. 4C) showed that A has been shifted by +3.7 (mV) from inactivation (Fig. 4C) showed that A has been shifted by +3.7 (mV) inactivation (Fig. 4C) showed that A has been shifted by +3.7 (mV).

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<td>k (mV)</td>
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<td>V_{1/2} (mV)</td>
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<td>−94.0 ± 1.6 *</td>
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<td>k (mV)</td>
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<td>V_{1/2} (mV)</td>
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<td>k (mV)</td>
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<tr>
<td>k (mV)</td>
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<td>−11.8 ± 1.2</td>
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<td>Development of slow inactivation</td>
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<tr>
<td>A</td>
<td>0.15 ± 0.02</td>
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<tr>
<td>τ (ms)</td>
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<td>322.3 ± 58.5</td>
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<td>Recovery from inactivation</td>
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<td>τ_r (ms)</td>
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<td>12.0 ± 1.4 *</td>
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<tr>
<td>τ_i (ms)</td>
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<tr>
<td>A_f</td>
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<td>0.67 ± 0.03</td>
</tr>
<tr>
<td>A_s</td>
<td>0.26 ± 0.04</td>
<td>0.30 ± 0.03</td>
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* p < 0.05 vs WT (Student’s t-test).
Recovery from inactivation (Fig. 4D) was markedly slowed in G1319V, due to an almost doubling of the fast recovery time constant ($p=0.03$, Table 1). In contrast, no significant differences in amplitude of fast ($A_f$) and slow ($A_s$) recovery components from inactivation were observed between WT and G1319V channels (Table 1). During sustained depolarization, cardiac sodium channels may enter a slow inactivated state, a conformational state distinct from fast inactivation, which requires a prolonged period of hyperpolarization to recover from [9,10]. Fig. 5A shows the time course of entry into the slow inactivated state for WT and G1319V. While the time constant ($\tau$) for the development of slow inactivation was not different for WT and G1319V, the fraction of channels ($A_s$) that entered the slow inactivated state was almost doubled for G1319V compared to WT ($p=0.02$, Table 1).

To study whether the mutation affected the slow and fast components of the inactivation gating differently, the voltage-dependence of fast (Fig. 5C) and slow (Fig. 5B) inactivation was examined separately. In Fig. 5B, $I_{Na}$ was expressed as the relative current ($P_2/P_1$) after a 1000-ms conditioning pulse to potentials between $-160$ and $-15$ mV, and a brief repolarization interval (20 ms) at $-120$ mV to allow for recovery from fast inactivation. In line with the enhanced slow inactivation described in Fig. 5A, nearly 40% of G1319V channels entered the slow inactivated state, but only $\sim 20\%$ of WT channels. In addition, the $V_{1/2}$ of slow inactivation was shifted by $-6.8$ mV in G1319V channels ($p=0.04$, Table 1). Finally, the slope factor $k$ of the slow inactivation curve was significantly larger for G1319V channels ($p=0.03$, Table 1), indicating a reduced sensitivity to voltage changes compared to WT channels. To study the voltage-dependence of fast inactivation (Fig. 5C), $Na_{1.5}$ channels were stimulated with a short conditioning pulse (20 ms) in order to avoid entrance into the slow inactivated state. Under these conditions, $V_{1/2}$ and the slope factor $k$ were similar for WT and G1319V channels (Table 1).
Therefore, we conclude that the negative shift in steady-state inactivation observed by using the protocol in Fig. 4C (with which effects on fast and slow inactivation cannot be distinguished), was exclusively due to changes in voltage-dependence of slow inactivation rather than to changes in the fast component. Thus, the G1319V mutation mostly affects slow inactivation.

The wider QRS intervals during exercise testing observed in G1319V carriers (Fig. 1) suggest a more prominent reduction in sodium channel availability at high heart rates for G1319V, which would be in line with the observed delay in recovery from inactivation (Fig. 4D) and the enhanced slow inactivation (Fig. 5A and B) [10]. To test this hypothesis, peak $I_{\text{Na}}$ was elicited by series of depolarizing steps, at pacing rates of 1 and 3 Hz (Fig. 6A). At 1 Hz, peak $I_{\text{Na}}$ remained unchanged in both WT ($n=8$) and G1319V ($n=12$). At 3 Hz, however, when the diastolic time was greatly reduced, G1319V ($n=9$) showed a significant decrease in peak $I_{\text{Na}}$ compared to WT ($n=5$) at all pulse numbers (Fig. 6B).

4. Discussion

We identified the DIIIS4–S5 linker of the $\alpha$-subunit of the cardiac sodium channel as a new region involved in slow inactivation by studying a novel Na$_1$.5 mutation, G1319V, which caused BrS. This mutation is located in the DIIIS4–S5 linker, a region previously linked to fast inactivation [13–15] and LQT3 [21–26]. Yet, we found no QT prolongation in carriers of this mutation, and no detectable $I_{\text{NaL}}$ in the mutant (Fig. 3D). Peak $I_{\text{Na}}$ amplitudes were similar for WT and G1319V mutant, indicating that both channel types are equally expressed at the plasma membrane. The mutation did however affect Na$_1$.5 gating properties, particularly inactivation. This involved a negative shift in voltage-dependence of inactivation, exclusively due to a negative shift of voltage-dependence of slow inactivation, slowing of the fast time constant of recovery from inactivation, and enhanced slow inactivation (Figs. 4 and 5, Table 1). All these gating changes contribute to a reduction in $I_{\text{Na}}$, in line with the proposed mechanism underlying BrS [4–7].
4.1. Comparison with other cardiac sodium channel mutations in the DIIIS4–S5 linker

Previous studies using site-specific antibodies and site-directed mutagenesis identified the linker between domains III and IV of the Na⁺ channel α-subunit as the region responsible for fast inactivation. The hydrophobic IFM motif is a putative inactivation particle which binds to the intracellular mouth of the pore via hydrophobic interactions and occludes it [11]. The DIIIS4–S5 linker is believed to be part of the inactivation gate receptor for the IFM motif [13–15]. This mechanism underlies fast inactivation of voltage-gated sodium channels throughout various tissues and species. In accordance with this critical role, the amino acid sequence of the DIIIS4–S5 linker has high evolutionary conservation amongst different human sodium channel isoforms and species (Fig. 2B). Previously published mutations located in the DIIIS4–S5 loop, N1325S, A1330P, A1330T, and P1332L (no electrophysiological characterization available) [21–26] are all associated with LQT3 (Fig. 2A). The G1319V mutation is the first reported mutation in the DIIIS4–S5 linker which causes BrS.

In N1325S (Fig. 2A), the partial disruption of fast inactivation causes \( I_{\text{NaL}} \), which is considered the main mechanism underlying LQT3 [8]. In A1330P and A1330T, \( I_{\text{NaL}} \) was absent, but the positive shift of inactivation caused...
QT prolongation by increasing window current during the repolarization phase of the AP. G1319V channels neither have detectable $I_{\text{Na},\text{st}}$, nor a positive shift of voltage-dependence of inactivation. In contrast, the negative shift of voltage-dependence of inactivation reduces Na$^+$ channel availability and window current, while the positive shift of activation contributed to a further reduction in $I_{\text{Na}}$ (elevated AP threshold, further reduction of window current). Moreover, the opposite changes in the rate of recovery from inactivation between G1319V (slowed) and A1330P/A1330T (hardened) contribute to opposite phenotypes. Finally, G1319V channels exhibit enhanced slow inactivation, as previously reported in other BrS-associated mutations (Table 2). This property was not investigated in the other mutations located in the DIIFS4–S5 linker.

While the positive shift in voltage-dependence of activation, and the negative shift in steady-state voltage-dependence of inactivation, conspire to reduce membrane excitability at baseline conditions, the delayed recovery from inactivation in concert with the enhanced slow inactivation, reduces channel availability at high heart rates due to accumulation of slow inactivation [10]. Accordingly, we observed an additional increase in QRS width up to 30% at fast heart rates during exercise in G1319V carriers (Fig. 1). This differs significantly from the general population, where QRS width is unchanged or even reduced during exercise testing [27,28]. QRS width, a measure of total cardiac activation time, is determined by heart size and conduction velocity [29]. As heart size is unchanged during exercise, increased QRS width is explained by reduced conduction velocity, potentially caused by reduced $I_{\text{Na}}$ magnitude. Accordingly, we found a marked reduction in peak $I_{\text{Na}}$ for G1319V channels compared to WT, at faster pacing rates (Fig. 6).

While QRS widening observed during exercise testing in G1319V carriers is thus explained by $I_{\text{Na}}$ reduction, it must be noted that lethal arrhythmias in most BrS patients occur at slow heart rates, notably during sleep [6]. This may indicate that increased slow inactivation and/or slowed recovery from inactivation are less common gating derangements than, for instance, decreased channel expression, in BrS-associated Na$_{\text{a},1.5}$ mutants. This notion, however, is unproven, as definitive proof requires analysis of slow inactivation/recovery from inactivation of all BrS-associated Na$_{\text{a},1.5}$ mutants.

### 4.2. The role of a glycine at position 1319 in the DIIFS4–S5 linker

The α-subunit of the Na$_{\text{a},1.5}$ channel is a complex molecule consisting of 4 homologous domains (DI–DIV), each composed of 6 membrane-spanning segments (S1–S6) linked by intracellular and extracellular loops (Fig. 2A). The linkers between S5–S6 (P-loops) control ion selectivity and permeation, while the positively charged segments S4 act as a voltage sensor, determining channel opening (activation) upon membrane depolarization. Finally, the DIII–DIV linker is responsible for channel fast inactivation [1,30]. However, unlike activation and fast inactivation, the molecular basis of slow inactivation is still a subject to debate. Serial mutagenesis and chimeric analysis suggest a key role of the P-loops that bend back into the membrane and

### Table 2

BrS, Brugada syndrome; LQT3, long QT syndrome type 3; PCCD, progressive cardiac conduction disease; $A$, fraction of channels that enter the slow inactivated state; $\tau$, time constant for development of slow inactivation; $V_{1/2}$, voltage of half-maximal (in)activation; $\tau_{\text{re}}$, fast time constant of recovery from inactivation; $I_{\text{peak}}$, sodium peak current; $I_{\text{Na},\text{st}}$, persistent sodium inward current; $\uparrow$, shift to negative voltage; $\downarrow$, shift to positive voltage; $\uparrow$, increase; $\downarrow$, reduction; $\equiv$, unchanged; $\nabla$, not reported

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<td>E1053K</td>
<td>DII–DIII</td>
<td>BrS</td>
<td>[35]</td>
<td>$\equiv$</td>
<td>$\equiv$</td>
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<td>$\equiv$</td>
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<td>ΔK1479</td>
<td>DIII–IV</td>
<td>BrS/PCCD</td>
<td>[38]</td>
<td>$\equiv$</td>
<td>$\equiv$</td>
<td>$\equiv$</td>
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<tr>
<td>K1527R/A1569P</td>
<td>DIV5/DIV2</td>
<td>BrS</td>
<td>[37]</td>
<td>$\equiv$</td>
<td>$\equiv$</td>
<td>$\equiv$</td>
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<tr>
<td>D1595N</td>
<td>DIV5</td>
<td>PCCD</td>
<td>[42]</td>
<td>$\equiv$</td>
<td>+4.2</td>
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<td>DIV3–S4</td>
<td>BrS</td>
<td>[9]</td>
<td>$\equiv$</td>
<td>$\equiv$</td>
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<tr>
<td>T1620M (32 °C)</td>
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<td>BrS</td>
<td>[9]</td>
<td>$\equiv$</td>
<td>$\equiv$</td>
<td>$\equiv$</td>
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<tr>
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<tr>
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All studies were performed at room temperature if not indicated otherwise.
Fig. 7. (A) Alignment of the residues 1262–1372 of SCN5A against the corresponding residues from the S3–S5 region of the human cyclic nucleotide gated channel alpha 1 (CNGA1) and the human potassium voltage-gated channel (KCNA2). "*" indicates identical residues, ':' indicates high similarity residues, and "." indicates similar residues. (B) Manually readjusted alignment of the transmembrane segment DIIIS4, the DIIIS4–S5 linker and transmembrane segment DIIIS5 of the SCN5A α-subunit based on the crystal structure of Kv1.2 α-subunit alignment. Symbols are as above. (C) Stereo-view of three-dimensional model representing the transmembrane segment DIIIS4, the DIIIS4–S5 linker and transmembrane segment DIIIS5 of the Na,1.5 α-subunit. (D) Enlargement of C representing the DIIIS4 segment and the DIIIS4–S5 linker. Arrows indicate the glycine at position 1319 in the DIIIS4–S5 linker and a phenylalanine at position 1317 in the DIIIS4 segment, respectively.
define the outer pore [30]. A second hypothesis involves a rearrangement of amino acid residues within the putative pore-lining S6 segments that would constrict the inner pore region [31,32]. These different findings may indicate that the correlation between Na1.5 mutation sites and channel gating properties is not always straightforward. Although a particular channel gating derangement might be directly linked to the position of the mutation, it is equally possible that the observed changes in gating properties occur through unknown interactions with other parts of the channel. Therefore, further delineation of the functional roles of the various Na1.5 regions is necessary for understanding channel functioning and its interaction with other proteins and drugs, since Na1.5 modulation may act both to suppress and facilitate life-threatening cardiac arrhythmias.

Here, we describe a novel BrS-associated SCN5A mutation which enhances slow inactivation. This finding was unexpected, since the G1319V mutation is located in a part of the channel so far exclusively related to fast inactivation [13–15]. As shown in Table 2, SCN5A mutations responsible for inherited arrhythmia syndromes, such as BrS, LQT3 and PCCD, which alter slow inactivation, are spread throughout several regions of Na1.5, with hot spots in the C-terminus, and the inner and outer pores of Na1.5 [7,9,10,33–44] (Table 2). This study defines the DIIIS4–S5 linker as another region involved in slow inactivation, therefore increasing our insights into structure–function relations of Na1.5.

In previously described LQT3-associated mutations located in the DIIIS4–S5 linker (Fig. 2A), impaired fast inactivation is probably due to the fact that these substitutions affect the secondary structure of the channel, thereby destabilizing the hydrophobic interaction between the IFM motif of the DIII–DIV linker and its receptor site (DIIIS4–S5 linker) [21–24]. In the mutant reported here, a glycine, a small amino acid which confers flexibility to polypeptides particularly at hinge regions, was replaced by a valine, an amino acid with a larger side chain (R-group). We speculate that such a substitution will perturb movement of the DIIIS4–S5 linker, necessary for channel inactivation, rather than disturbing the actual structure of this channel segment. When the S4 segments move upon depolarization, the S3–S4 and S4–S5 segments, coordinated to the N-terminal and C-terminal ends of S4, respectively, must be able to accommodate S4 displacement. This movement exposes the binding site for the DIII–DIV linker and allows channel inactivation [45]. Fig. 7D represents the stereo-view of the three-dimensional structure of the DIIIS4 segment and the DIIIS4–S5 linker of the α-subunit of Na1.5. The model offers the postulation that the presence of the large aromatic ring of phenylalanine 1317 at the terminal end of DIIIS4 can presumably only be accommodated by the presence of a small amino acid in the helical loop junction at position 1319. A valine rather than glycine, at position 1319 will potentially create a steric hindrance to the movement of the DIIIS4–S5 linker, thus destabilizing the inactivation property, particularly slow inactivation, of the channel. Since valine presents an uncharged hydrophobic R-group, additional electrostatic interaction with the positively charged DIIIS4 voltage sensor is unlikely.

5. Summary

Characterization of a novel BrS-associated SCN5A mutation, G1319V, revealed the DIIIS4–S5 linker of Na1.5 as a new region involved in slow inactivation. Electrophysiological changes in the properties of fast and slow inactivation of G1319V channels are responsible for conduction slowing at fast heart rates and the BrS phenotype.

Acknowledgements

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References


