Brugada syndrome and fever: Genetic and molecular characterization of patients carrying SCN5A mutations

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Abstract

Objective: Brugada syndrome (BrS) is characterized by ventricular tachyarrhythmias leading to sudden cardiac death and is caused, in part, by mutations in the SCN5A gene encoding the sodium channel Na1.5. Fever can trigger or exacerbate the clinical manifestations of BrS. The aim of this work was to characterize the genetic and molecular determinants of fever-dependent BrS.

Methods: Four male patients with typical BrS ST-segment elevation in V1–V3 or ventricular arrhythmias during fever were screened for mutations in the SCN5A gene. Wild-type (WT) and mutant Na1.5 channels were expressed in HEK293 cells. The sodium currents (INa) were analysed using the whole-cell patch clamp technique at various temperatures. Protein expression of WT and mutant channels was studied by Western blot experiments.

Results: Two mutations in SCN5A, L325R and R535X, were identified. Expression of the two mutant Na1.5 channels in HEK293 cells revealed in each case a severe loss-of-function. Upon the increase of temperature up to 42°C, we observed a pronounced acceleration of Na1.5 activation and fast inactivation kinetics. Cardiac action potential modelling experiments suggest that in patients with reduced INa, fever could prematurely shorten the action potential by virtue of its effect on WT channels. Further experiments revealed that L325R channels are likely misfolded, since their function could be partially rescued by mexiletine or curcumin. In co-expression experiments, L325R channels interfered with the proper function of WT channels, suggesting that a dominant negative phenomenon may underlie BrS triggered by fever.

Conclusions: The genetic background of BrS patients sensitive to fever is heterogeneous. Our experimental data suggest that the clinical manifestations of fever-exacerbated BrS may not be mutation specific.

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1. Introduction

Brugada syndrome (BrS) is characterized by ST segment elevation in the right precordial leads V1–V3 on the surface ECG, with incomplete or complete right bundle branch block and an increased risk for sudden cardiac death (SCD) caused by polymorphic ventricular tachycardia or fibrillation (VT/VF) [1]. The BrS is a primary electrical cardiac disorder with no apparent underlying structural heart disease and is inherited by an autosomal dominant trait. Mutations have been identified in the SCN5A gene encoding the α-subunit of the voltage-gated sodium channel Na\(_{\alpha}1.5\) [2]. Yet, only 10–30% of clinically affected individuals carry a mutation in this gene [3]. Most of SCN5A mutations lead to a “loss-of-function” by reducing the sodium current (I\(_{\text{Na}}\)) available during the phases 0 (upstroke) and 1 (early repolarization) of the cardiac action potential (AP) [1].

The molecular and cellular mechanisms leading to the BrS are not yet completely understood. In some patients, the BrS is concealed on the surface ECG and can be unmasked using sodium channel blockers. Furthermore, fever has been reported to unmask or exacerbate the typical BrS ECG pattern on the surface ECG and to trigger VT [4]. During the past six years, several case reports of fever-induced BrS have been published [5–9].

In the present study, we screened SCN5A in four unrelated patients presenting typical BrS ECGs during episodes of fever. Two heterozygous mutations were identified: a novel missense mutation, L325R, and a nonsense mutation, R535X. We carried out molecular and functional analyses of the consequences of these two mutations, using classical biochemical and patch-clamp techniques. The two mutations markedly decreased the I\(_{\text{Na}}\) after transfection of the mutant proteins, suggesting that reduced availability of I\(_{\text{Na}}\) during the early phase of the cardiac AP may cause fever-induced tachyarrhythmias. We also provide experimental evidence for protein misfolding resulting from the L325R mutation, since the I\(_{\text{Na}}\) mediated by these mutant channels was partially rescued by curcumin and mexiletine. In addition, we present data suggesting for the first time that BrS mutant channels may exert a dominant negative effect on WT channels.

2. Methods

This study was performed according to the guidelines of the Swiss Society of Medical Genetics and conforms to the Declaration of Helsinki. All individuals gave written informed consent to participate in the study.

2.1. Molecular screening

Genomic DNA was extracted from peripheral lymphocytes. In the four patients, the coding exons of SCN5A were amplified by polymerase chain reaction (PCR), using primers designed in intronic flanking sequences, according to the gene sequences [10]. Denaturing high performance liquid chromatography (DHPLC) was performed on DNA-amplification products in at least one temperature condition. Abnormal DHPLC profiles were analysed by sequence reaction in both strands of the exon, using big dye termination mix, and analysed by cycle sequencing on an automated laser fluorescent DNA sequencer (ABI prism 3100, Applied Biosystems). The novel mutation L325R was absent in 200 normal alleles.

2.2. Functional and biochemical analysis of Na\(^{+}\) channels

SCN5A mutations were engineered into WT cDNA (clone hH1a received from Dr. R. Kass) cloned in pcDNA3.1 (Invitrogen), using the QuickChange Kit (Stratagene) and verified by sequencing. For electrophysiology studies, HEK293 cells were transiently transfected with 0.6 μg WT or mutant Na\(_{\alpha}1.5\) construct cDNAs or as indicated. All transfections included 2.0 μg pIREs-h\(\beta\)1-CD8 cDNA encoding h\(\beta\)1 subunit and CD8 antigen, as a reporter gene. Cells were transfected using calcium phosphate and I\(_{\text{Na}}\) were measured after 48 h. Anti-CD8 beads (Dynal) were used to identify transfected cells. Patch-clamp recordings were carried out using an internal solution containing (mmol/L) CsCl 60; Cs Aspartate 70; EGTA 11; MgCl\(_2\) 1; CaCl\(_2\) 1; HEPES 10; and Na\(_2\)-ATP 5, pH 7.2 with CsOH; external solution NaCl 130; CaCl\(_2\) 2; MgCl\(_2\) 1.2; CsCl 5; HEPES 10; and glucose 5, pH 7.4 with CsOH. Using these solutions, 5 min after rupturing the membrane, we observed no significant alteration of the availability curve and the peak current. Holding potentials were −100 mV and no leak subtraction was performed. Peak currents were measured during a current-voltage protocol and I\(_{\text{Na}}\) densities (pA/pF) were obtained by dividing the peak I\(_{\text{Na}}\) by the cell capacitance obtained from the pClamp function. Measurements were carried out at different temperatures using a control system (Cell MicroControls) heating the perfusion solution. Obtaining an adequate voltage-clamp control when measuring voltage-gated I\(_{\text{Na}}\) in voltage-clamp conditions at physiological temperatures may represent a difficult task. We took great care to ensure the best possible voltage-clamp control: (1) We compensated the series resistances by virtually 100% using a VE-2 amplifier (Alembic Instruments) based on a “state estimator” series resistance compensation approach [11]. (2) The resistance of the pipettes was in the range of 1.7–2.5 MΩ. (3) We used data only from cells where the access resistance remained stable over all the duration of the experiment. (4) Cells for which signs of poor voltage-clamp control such as delayed inflections of the current or discontinuities in the peak I\(_{\text{Na}}\) vs. Vm curve were not analysed. As presented in the Table 1, despite the fact that activation kinetics were faster with increasing temperature (22 °C up to 42 °C, see Fig. 4) the voltage dependence of activation characterized by the V\(_{1/2}\) of activation and, most importantly, the slope factor K were
not significantly influenced by the temperature, indicating that the quality of the voltage-clamp control was comparable at all temperatures tested. In some experiments, cells were pre-incubated with thapsigargin (Acros), curcumin (Acros), or mexiletine (Sigma). The cells were washed of the drugs 10 min prior to the recordings.

Western blotting conditions have been described previously [12]. The anti-Na\textsubscript{a},1.5 affinity purified antibody recognizes residues 493–511 of rat Na\textsubscript{a},1.5 (ASC-005, Alomone). These residues are identical in the human sequence. Anti-actin antibody was from Sigma.

Data are presented as means±SEM. Two-tailed Student t-test was used to compare means; p < 0.05 was considered significant.

2.3. Mathematical modelling of \( I_{\text{Na}} \) and of the action potential

WT \( I_{\text{Na}} \) was modelled using the 6-state Markovian model of Clancy and Rudy [13] (errata on http://rudylab.wustl.edu/research/cell/methodology/markov/ina.htm). Temperature-dependence of the transition rates was incorporated using a \( Q_{10} \) factor approach according to the following equation:

\[
\alpha(T) = Q_{10}^{(T - T_0)/10} \alpha(T_0),
\]

where \( T \) is absolute temperature, \( \alpha(T) \) is the rate at temperature \( T \), and \( \alpha(T_0) \) the original model rate at the reference temperature \( T_0 \) of 310.15 \( ^\circ \)K. To reconstruct the AP of the right ventricular epicardium, this \( I_{\text{Na}} \) formulation was introduced into the Luo–Rudy dynamic (LRd) cell model [14], with the updates proposed by Faber [15]. As done previously [16], the transient outward \( K^+ \) current \( (I_{\text{to}}) \) was incorporated according to Dumaine et al. [17] with a maximal conductance of 1.35 mS/\muF to simulate the high \( I_{\text{to}} \) density in the right ventricular epicardium. The AP was elicited by a 0.5 ms current pulse of 80 \muA/\muF.

3. Results

3.1. Description of four index cases with fever-sensitive BrS

This study included four male index cases of Caucasian origin in whom either typical BrS ECG pattern and/or VT was triggered by fever. Patient 1, 44-year-old, was admitted in the emergency room for investigation of a first episode of syncope during fever (up to 39 \( ^\circ \)C) caused by pneumonia. The ECG was suggestive of BrS (Fig. 1) and an ICD was implanted. During three fever episodes due to pneumonia, the ECG showed a typical BrS pattern (Fig. 1). Two days later, when the patient was afebrile, the ECG was normal. A flecainide test was positive, but no ventricular arrhythmia was inducible during EPS. The clinical presentation of the fourth patient, a 50-year-old
male, has been previously published [5]. Briefly, the patient was admitted at the hospital with pleuritis and fever (38.1 °C). The ECG showed downward sloping ST segment elevations in V1–V3 and was normalized as soon as the patient was afebrile. However, a similar abnormal ECG was recorded after infusion of ajmaline. Only patient 3 had a family history of SCD; his half-brother died suddenly at age 48 for an unknown reason, with no demonstrable heart disease.

3.2. Identification of two SCN5A mutations

We screened all coding exons of SCN5A by a PCR-DHPLC-sequencing approach. A novel heterozygous missense mutation was identified in patient 2. A T to G substitution at position 974 resulted in the L325R mutation with a positively charged arginine (Fig. 2A). The leucine-325 residue is located in the linker segment preceding the domain 1 P-loop (Fig. 2C). In patient 3, a heterozygous non-sense mutation was identified, R535X, resulting from a C to T substitution at position 1603. This mutation leads to a premature stop codon (Fig. 2B) and has been previously described [18]. However, an investigation of the functional consequences of this mutation has not been performed, yet.

3.3. Electrophysiological characterization of the two mutant Na$_{\text{v}}$1.5 channels

The functional consequences of the SCN5A mutations were studied, using HEK293 cells transiently transfected with mutant cDNAs. As illustrated in Fig. 3A, the level of expression of the L325R mutant channels analysed by Western blot was comparable to that of WT channels. In contrast, using the patch-clamp technique, L325R channels generated $I_{\text{Na}}$ that was reduced by about 80%, compared to WT channels (Fig. 3B–C). At room temperature (22 °C), the steady-state inactivation parameters of L325R channels were not different from WT (Table 1). In contrast, the L325R mutation caused a ~10 mV positive shift of the $V_{1/2}$ steady-state activation curve (Table 1). Kinetics of activation (assessed by the time to the peak) and onset of fast inactivation were slower for the L325R $I_{\text{Na}}$ (Table 1 and Fig. 4A).
Transient expression of R535X channels yielded a truncated protein with a molecular weight which, as predicted from the sequence, was about one fourth of that of the WT protein (Fig. 3D). No $I_{\text{Na}}$ could be recorded from cells expressing the truncated protein (Fig. 3E–F).

### 3.4. Effect of temperature on the properties of WT and L325R channels and on the action potential

In order to get further insight into the mechanisms underlying the effect of fever, we analysed the effect of temperature on WT and L325R channels. Data presented in the Table 1 (and see Methods) illustrates that we achieved an excellent voltage-clamp control for the recording of $I_{\text{Na}}$ at physiological temperatures. Increasing the temperature significantly accelerated both the activation and inactivation kinetics of WT and L325R $I_{\text{Na}}$ (Fig. 4A and Table 1) and, in agreement with a previous study [19], the resultant peak $I_{\text{Na}}$ was increased. The peak WT $I_{\text{Na}}$ was 16±4% larger at 42°C compared to 22°C; L325R $I_{\text{Na}}$ was increased by 24±8%. The steady-state inactivation and activation parameters were also measured at 37°C and 42°C and the difference in activation $V_{1/2}$ that was observed at room temperature was still present (Table 1). No significant temperature-dependence of both $V_{1/2}$ and $\kappa$-slope factor was observed, neither for activation, nor for inactivation.

How to explain the increase in peak $I_{\text{Na}}$ paralleling the increasing temperature? If one assumes that all kinetic processes governing $I_{\text{Na}}$ follow an identical sensitivity to temperature ($Q_{10}$ factor), one would expect $I_{\text{Na}}$ to exhibit an overall accelerated time course without any major change of its peak. This increase of peak $I_{\text{Na}}$ could thus be the consequence of a $Q_{10}$, which is larger for activation than for inactivation, leading to a relatively larger acceleration of activation, compared to inactivation. To test this hypothesis, we used, in a first step, a Hodgkin–Huxley formalism to estimate $Q_{10}$ for activation and inactivation by fitting WT $I_{\text{Na}}$ recordings elicited by pulses to −10 mV with the function $I_{\text{Na}} = g_{\text{Na}}I_{\text{Na}}^\text{max} \cdot (1 - \exp(-\alpha_m t)) \cdot (\exp(-\alpha_h t)) \cdot (V - E_{\text{Na}})$, where $\alpha_m$ and $\alpha_h$ are the rate constants for activation and inactivation, respectively. Then, the $Q_{10}$ factors were computed from the slope of the linear regression of $\ln(\alpha_m)$ and $\ln(\alpha_h)$ as a function of temperature. $Q_{10}$ values of 2.11 ($r=0.97$) and 1.90 ($r=0.99$) were obtained for $\alpha_m$ and $\alpha_h$, respectively. In a second step, we
reconstructed $I_{\text{Na}}$, as shown in Fig. 4B and C, using a Markovian model of the WT channel [13], in which we incorporated temperature-dependence using the $Q_{10}$ formalism. When we assumed $Q_{10}$ to be identical for all rate constants (the value of 2.0 was chosen between 2.1 and 1.9), increasing temperature from 22 to 42 °C resulted only in a modest peak $I_{\text{Na}}$ increase of 5.2% (Fig. 4B). In the simulation presented in Fig. 4C, we incorporated $Q_{10}$ values of 2.15 for all three activation transitions and 1.9 for both inactivation transitions. The absence of a temperature-dependence of $V_{1/2}$ for steady-state activation and availability in our experiments indicates that there is no change in the steady-state occupancy of the different states with increasing temperature. Consequently, this suggests that for a given transition, the ratio of the forward and backward rates does not change with temperature. Therefore, the $Q_{10}$’s for the reverse transitions (deactivation and recovery from inactivation, respectively) were set equal to that of the forward transitions. The $Q_{10}$’s for the transition between states IF and C1 could not be determined from our experiments and an intermediate value of 2.0 was assumed in both directions. Using this set of $Q_{10}$ parameters, peak $I_{\text{Na}}$ increased by 24.8% when temperature was increased from 22 to 42 °C (Fig. 4C), an increase which is similar to that observed experimentally. The effects of this temperature-dependent behaviour of WT $I_{\text{Na}}$ on the right ventricular epicardial AP were further investigated using the LRd model (Fig. 5). In the control simulation (100% $I_{\text{Na}}, 37 °C$, Fig. 5A), the AP exhibited a deep notch characteristic of the right ventricular AP. No significant changes in AP morphology were observed when either temperature was increased to 40 °C (Fig. 5B) or when $I_{\text{Na}}$ was reduced to 50% to simulate a heterozygote BrS mutant cell (Fig. 5C). However, with 50% $I_{\text{Na}}$ and at 40 °C, the AP exhibited premature repolarization and a loss of the dome, consistent with the cellular model of BrS (Fig. 5D).

3.5. Rescue of the L325R Na,1.5 currents

The large decrease in peak current density caused by the L325R mutation (about 80%) cannot be explained by the alterations in the biophysical properties of L325R Na,1.5.
Because the mutation replaces an uncharged leucine by a positively charged arginine, we considered the hypothesis that this mutation influences the folding process of the protein, thus altering either biosynthesis or trafficking of the channel. Fig. 6A illustrates that, similarly to a recent report [20], pre-incubation of the cells expressing L325R channels with 500 μM mexiletine rescued the mutant I_{Na} up to 55% of the WT current. We also investigated the effect of curcumin, which has been shown to rescue misfolded mutant CFTR channels in a mouse model of cystic fibrosis [21]. This drug is a low-toxic sarcoplasmic reticulum Ca^{2+}-ATPase inhibitor [22]. Incubation with 25 μM of curcumin during 24 h increased significantly the mutant currents by ¨150% (Fig. 6A). In contrast, thapsigargin, another drug known to rescue certain misfolded ion channels [23], had no effect. In addition to these treatments expected to improve trafficking of misfolded channels, we also incubated cells expressing L325R channels for 24 h at 28˚C. This condition also partially rescued the mutant channels by ¨300% (Fig. 6A).

3.6. Dominant negative effect of the L325R mutant allele

Consistent with an autosomal dominant trait, both SCN5A mutation carriers were found to be heterozygous. In order to mimic this state in our cellular model, we investigated the co-expression of WT and mutant “alleles” by co-transfecting WT and mutant cDNAs. Fig. 6B shows that when reducing the amount of WT cDNA by 50%, the resultant I_{Na} was decreased by about 50%. This condition represents the clinical situation where there would be no synthesis at all of the protein resulting from the mutant allele. However in our experimental model, both mutants were expressed (Fig. 3A and D). When WT and L325R channels were co-transfected in a 1:1 ratio, the peak I_{Na} was reduced to only 18±8% of the control condition where 100% of WT channels were expressed (Fig. 6B). This finding strongly suggests that the L325R allele exerts a dominant negative effect on WT channels. In contrast, this negative effect was not observed when the truncated R535X
and the WT proteins were co-transfected (Fig. 6B). Since, the total pool of expressed Na\(_{\text{1.5}}\) proteins is unchanged (Fig. 6C), it can be postulated that the L325R channels interfere with the trafficking of WT channels.

4. Discussion

In this study, we identified two mutations in the SCN5A gene in four index patients with BrS pattern ECGs during episodes of fever. Our in vitro data indicate that the mutations cause an important loss-of-function of the Na\(_{\text{1.5}}\) channels. Functional analyses of the L325R and R535X mutant channels suggest that the manifestations of fever-exacerbated BrS may not be mutation specific. Furthermore, our experiments suggest novel molecular mechanisms underlying BrS, such as misfolding of mutant proteins leading to dominant negative effect of mutant channels.

4.1. Clinical and genetic findings

Fever can trigger VT/VF in BrS patients [4]. Here, we report four male cases in which SCN5A has been screened. Two mutations were found: R535X in an asymptomatic patient with a typical BrS ECC pattern during fever and L325R, a novel mutation, in a patient with fever-induced VT. The main clinical implications of these findings are that BrS has to be considered in patients with unexplained syncope during febrile state, and that genetic investigation should be performed in patients with an ECG suggestive of BrS during fever. Finally, BrS patients should be advised to take antipyretic drugs early in the course of any febrile illness.

4.2. Possible mechanisms of fever susceptibility in BrS patients

One of the current models explaining the ECG alterations seen in BrS is based on an imbalance between the depolarizing and repolarizing currents during the AP phase 1, mainly in cells expressing a large transient outward \(I_{\text{to}}\) current as the epicardial cells of the right ventricle [1]. In patients with loss-of-function mutations of Na\(_{\text{1.5}}\), resulting in less \(I_{\text{Na}}\) during the phase 1, the large \(I_{\text{to}}\) current may repolarize the membrane prematurely producing a loss of the dome (phase 2) of the AP. When such premature AP shortening happens heterogeneously in the myocardium, this may generate phase 2 re-entries that can cause VT/VF. Hence, the delicate balance of currents, mainly \(I_{\text{to}}\) (repolarizing), \(I_{\text{Ca}}\) and \(I_{\text{Na}}\) (both depolarizing), during the AP phase 1 is very critical.

How could an increase of temperature above 37 °C alter this subtle balance in patients? It has been shown that the T1620M SCN5A mutation found in patients with “classical” BrS alters the temperature sensitivity of fast inactivation of these channels [17]. However, our study suggests that in patients carrying a SCN5A mutation at the heterozygous state in whom the function of mutant channels is either reduced or abolished, the temperature-dependent properties of WT \(I_{\text{Na}}\) itself might also lead to the typical ECG characteristics during fever. This concept is supported by the observation that the patient with the R535X mutation (channels yielding no measurable current) and the patient with the L325R mutation (channels mediating only a small \(I_{\text{Na}}\)) both exhibited sensitivity to fever. Moreover, the investigation of the L325R channel properties at different temperatures was not consistent with a mutation specific effect of temperature that would lead to a further decrease of mutant \(I_{\text{Na}}\). In consequence, we postulate that the effect of elevated temperature on the remaining \(I_{\text{Na}}\), which is mainly or totally mediated by WT channels, could be responsible for the ECG phenotype in BrS patients. Fig. 4 illustrates that the effects of increasing the temperature on \(I_{\text{Na}}\) are multiple and intricate, suggesting that distinct temperature sensitivities govern the different state transitions of the sodium channel (e.g., activation and inactivation). As supported by the simulations presented in Fig. 5, this complex temperature-dependence of WT \(I_{\text{Na}}\) might then be crucial in determining the balance of currents during depolarization and during the phase 1 of the AP. During phase 1, most of \(I_{\text{Na}}\) has undergone fast inactivation and only a small fraction of \(I_{\text{Na}}\) is still contributing to depolarization. Although small, our simulations indicate that this fraction is nonetheless critical. Therefore, accelerated inactivation under conditions of elevated temperature, combined with a decreased level of expression of Na\(_{\text{1.5}}\) channels, might then be sufficient to obliterate the “phase 1 depolarization reserve” and to shift the delicate balance of currents in favour of a premature repolarization, thus revealing a BrS phenotype. This hypothesis is supported by a recent model study by Gima and Rudy who demonstrated that the accelerated inactivation of the T1620M Na\(_{\text{1.5}}\) channel leads to a BrS ECG phenotype through a decreased \(I_{\text{Na}}\) [16]. However, since the differences in WT \(I_{\text{Na}}\) properties measured at 37 and 42 °C were minimal (Table 1), one should be cautious in extrapolating our experimental data to the situation of BrS patients with fever. Furthermore, the influence of elevated temperature on the AP is complex and the role of fever in BrS is likely to be multifactorial. It is plausible that temperature-dependent changes of currents other than \(I_{\text{Na}}\) could precipitate the BrS during fever. For example, fever may also increase the peak transient outward current (\(I_{\text{to}}\)) through mechanisms similar to those we propose for \(I_{\text{Na}}\) and precipitate repolarization in epicardial tissue. It was beyond the scope of this study to introduce temperature-dependent kinetics for currents other than \(I_{\text{Na}}\) and we are aware that this represents a limitation of the model; however, it allows us to demonstrate that the mechanism we propose may be a realistic option that remains to be explored further.
4.3. Misfolding of Na\textsubscript{v}1.5 protein contributes to the L325R phenotype

The consequences of the BrS SCN5A mutations on Na\textsubscript{v}1.5 function are multiple [1]. Recently, Chahine’s group reported three BrS SCN5A missense mutations leading to a charge modification and generating channels that were not trafficked to the membrane [24–26]. The L325R mutation described here leads to a similar phenotype since the protein expression was not altered (Fig. 3A) and treatments with chemical chaperones, as well as expression at 28 °C, partially rescued I\textsubscript{Na}. Misfolding of Na\textsubscript{v}1.5 therefore appears to be one of the mechanisms underlying BrS, as it is the case for K\textsubscript{v}LQT1 and hERG channels in congenital long QT syndrome [27], and CFTR channels in cystic fibrosis [21]. The significant mutant I\textsubscript{Na} increase obtained by treating the cells with curcumin may be of clinical importance since this drug has a very low toxicity profile and has already been tested in a clinical setting as an anti-cancer drug [28]. In contrast, thapsigargin, which similarly to curcumin can block the endoplasmic reticulum Ca\textsuperscript{2+}-pump, did not rescue the mutant I\textsubscript{Na}. It may therefore be possible that the positive effect of thapsigargin is restricted for specific channels [23].

4.4. Dominant negative effect of mutant Na\textsubscript{v}1.5

A dominant negative effect of mutant ion channel subunits has been frequently reported for channels formed by multiple α-subunits, such as the K\textsubscript{v}LQT1 channel in LQTS [29]. In contrast, Na\textsubscript{v}1.5 is not known to oligomerize with other α-subunits in order to form a minimal ion channel, and, therefore, the dominant negative effect of L325R channels on WT Na\textsubscript{v}1.5 (Fig. 6B) was unexpected. It can be postulated that mutant proteins interfere directly or indirectly with either biosynthesis or trafficking of WT channels. It also remains to be shown whether this mechanism could be generalized to other mistrafficked BrS channels since, to our knowledge, no study directly addressed this issue.

5. Conclusions

In the past few years, a number of case reports described that fever triggers the clinical manifestations of BrS. Hence, based on these studies and the present work, BrS should be considered in any patient with syncope during febrile state. The genetic background of BrS is heterogeneous. In the small population of fever-susceptible cases presented here, two patients out of four did not carry any mutation in SCN5A. Additional investigations are therefore needed to identify the genetic bases of this disorder. Finally, as illustrated by our findings, the molecular and cellular mechanisms underlying the fever-dependent manifestations of BrS are complex. Further studies are needed in order to elucidate this important issue since there is no curative treatment available for BrS to date.

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