A novel *SCN5A* mutation associated with idiopathic ventricular fibrillation without typical ECG findings of Brugada syndrome

Jun Akai[a,b,1], Naomasa Makita[d,1], Harumizu Sakurada[e], Nobumasa Shirai[d], Kazuo Ueda[b], Akira Kitabatake[d], Kiyoshi Nakazawa[f], Akinori Kimura[a,b], Masayasu Hiraoka[a,c,*]

[a] *Etiology and Pathogenesis Research Unit, Medical Research Institute, Tokyo Medical and Dental University, Yushima 1-5-45, Bunkyo-Ku, Tokyo 113-8510, Japan*
[b] *Department of Molecular Pathogenesis, Medical Research Institute, Tokyo Medical and Dental University, Yushima 1-5-45, Bunkyo-Ku, Tokyo 113-8510, Japan*
[c] *Department of Cardiovascular Diseases, Medical Research Institute, Tokyo Medical and Dental University, Yushima 1-5-45, Bunkyo-Ku, Tokyo 113-8510, Japan*
[d] *Department of Cardiovascular Medicine, Hokkaido University Graduate School of Medicine, Sapporo, Japan*
[e] *Department of Cardiology, Tokyo Metropolitan Hiroo Hospital, Tokyo, Japan*
[e] *Department of Internal Medicine, Division of Cardiology, St. Marianna University, School of Medicine, Kanagawa, Japan*

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Abstract Mutations in the human cardiac Na^+ channel α subunit gene (*SCN5A*) are responsible for Brugada syndrome, an idiopathic ventricular fibrillation (IVF) subgroup characterized by right bundle branch block and ST elevation on an electrocardiogram (ECG). However, the molecular basis of IVF in subgroups lacking these ECG findings has not been elucidated. We performed genetic screenings of Japanese IVF patients and found a novel *SCN5A* missense mutation (S1710L) in one symptomatic IVF patient that did not exhibit the typical Brugada ECG. Heterologously expressed S1710L channels showed marked acceleration in the current decay together with a large hyperpolarizing shift of steady-state inactivation and depolarizing shift of activation. These findings suggest that *SCN5A* is one of the responsible genes for IVF patients who do not show typical ECG manifestations of the Brugada syndrome.

Key words: Sodium channel; *SCN5A*; Sudden cardiac death; Ventricular fibrillation; Brugada syndrome; Electrophysiology

1. Introduction

Idiopathic ventricular fibrillation (IVF) is a syndrome that causes sudden cardiac death in individuals with an apparently normal heart [1]. Brugada syndrome is defined as an IVF characterized by right bundle branch block (RBBB) and ST segment elevation in leads V_1–V_3 on an electrocardiogram (ECG) and a hereditary occurrence is suggested in some patients [2]. A responsible gene for Brugada syndrome genetically demonstrated so far is the cardiac Na^+ channel α subunit gene (*SCN5A*), and several missense mutations including T1620M, splice-donor, frameshift mutations [3], and an insertion mutation [4] have been identified.

Despite significant insight into pathophysiology of IVF provided by recent molecular biological studies, there are issues remain to be elucidated. ST elevation of ECG in Brugada syndrome patients tends to fluctuate with time during follow-up, and the underlying precise mechanisms are still obscure although the autonomic nervous system is considered to play pivotal roles in this phenomenon. Furthermore, it has been clinically recognized that there is at least another subgroup in IVF that lacks electrophysiological manifestations characterized for the Brugada syndrome. It is not clear, therefore, whether these subgroup patients have mutations in *SCN5A* with subtle biophysical abnormalities, or alternatively, they may have a genetic basis distinct from the Brugada syndrome.

In order to address these issues, we have genetically screened IVF patients of both subgroups with and without typical ECG findings of Brugada syndrome. A novel missense mutation (S1710L) in *SCN5A* was found in a symptomatic patient of the latter group. Heterologously expressed S1710L mutant Na^+ channel showed alterations in channel inactivation and activation that could potentially result in a decreased Na^+ current amplitude.

2. Materials and methods

2.1. Subjects

Twenty-five Japanese IVF patients were genetically screened with informed consent. A clinical diagnosis of IVF was made based on the symptoms of at least one episode of syncope and/or cardiac arrest and documentation of ventricular fibrillation (VF). Structural heart diseases were excluded by chest X-ray, exercise ECG, echocardiogram, ventriculography, and coronary angiography. Eighteen patients were diagnosed as Brugada syndrome [2].

A 39-year-old man VF06, whose *SCN5A* mutation was further examined in this study, was admitted to the hospital because of recurrent syncope. His paternal grandfather and a paternal uncle had died suddenly in their sixth decade of unknown cause, however, paternal parents and siblings of the patient were asymptomatic. ECG on admission showed a first degree A-V block and marginal QRS prolongation (0.10 s) without ST elevation (Fig. 1A), but changed to complete RBBB with junctional ST elevation in V_1 (Fig. 1B) with increase in heart rate. However, coved or saddleback type ST elevations in V_{1-3}, typical ECG patterns characterized for Brugada syndrome, have never been documented even during therapy with disopyramide. He had an episode of spontaneous VF attack in the

*Corresponding author. Fax: (81)-3-5684 6295.
E-mail: hiraoka.card@mri.tmd.ac.jp

[1] These authors contributed equally to this work.

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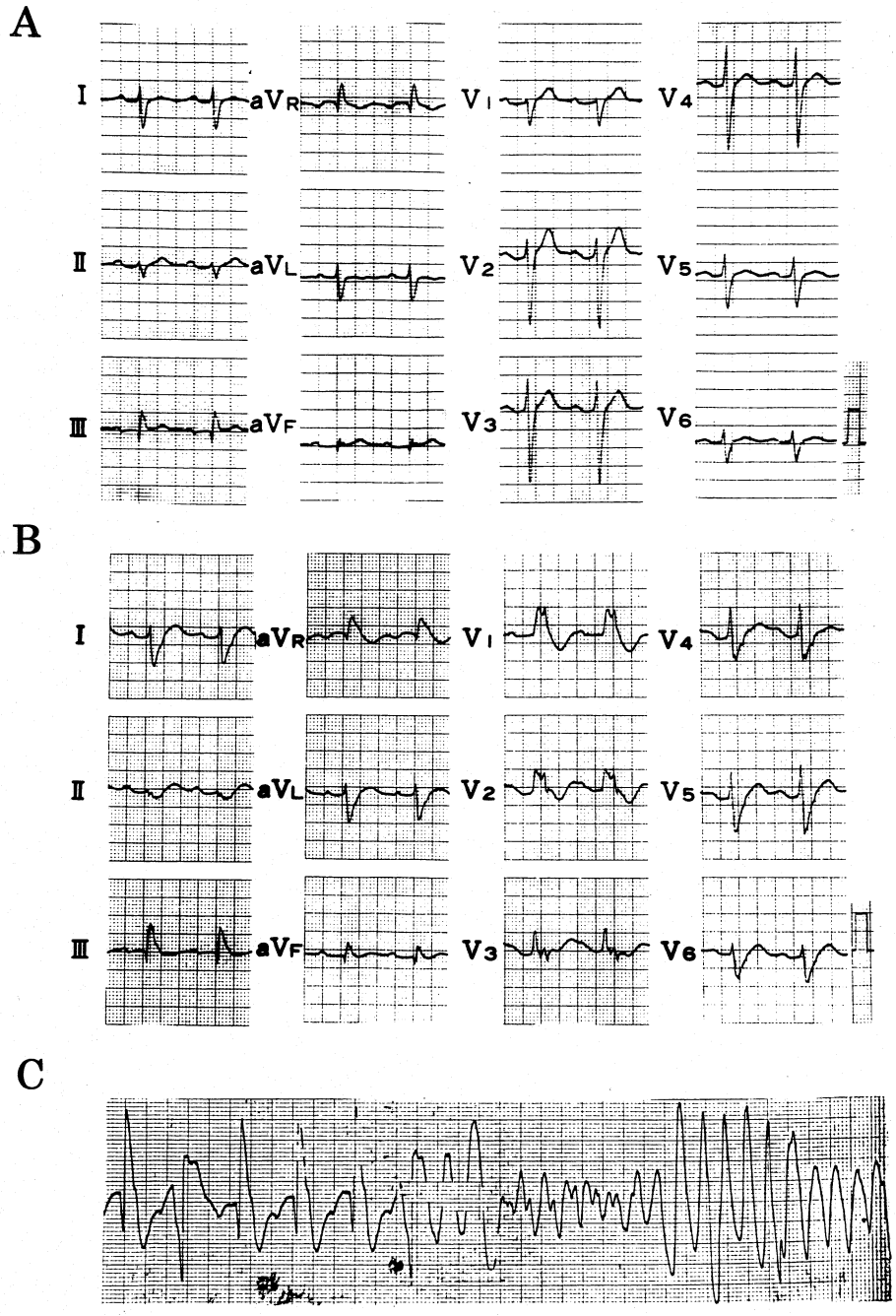

Fig. 1. ECG of the patient VF06. A: ECG on admission shows no ST elevation nor RBBB but marginal QRS prolongation to 0.10 s. B: Junctional ST elevation with complete RBBB with mild tachycardia was observed 2 h after admission. C: Monitor tracings exhibiting the onset of polymorphic VT later degenerated into VF and terminated by DC shock.

hospital that was aborted by defibrillation (Fig. 1C). Clinical electrophysiological study showed H-V prolongation, and VF was inducible by burst pacing from the right ventricular apex. After a cardiac defibrillator was prophylactically implanted, he had no palpitation nor syncope. The proband has no children and other family members did not agree with further examinations including DNA diagnostics.

2.2. Polymerase chain reaction-DNA conformation polymorphisms (PCR-DCP) analysis and DNA sequencing

Genomic DNA was extracted from peripheral blood leukocytes. All 28 exons of *SCN5A* were amplified by PCR using primers designed by Wang et al. [5], and analyzed by PCR-DCP as reported previously [6]. The PCR product showing an aberrant conformer was subcloned into pCR2.1 (Invitrogen) and multiple independent clones were sequenced using ABI 373 DNA sequencer (Applied Biosystems).

2.3. Functional expression

Site-directed mutagenesis of the human heart sodium channel α subunit (hH1) was performed as described [7]. S1710L mutant and wild type (WT) cDNAs were subcloned into pRcCMV plasmid (Invitrogen). Human embryonic kidney cells stably expressing human Na^+ channel β_1 subunit (HEK-hβ_1) [8] were transiently transfected with either WT or S1710L cDNA. Cells were cotransfected with a plasmid encoding CD8 to visually identify cells expressing heterologous a subunit cDNA with Dynabeads M-450 CD8 (Dynal). Na^+ currents were recorded using the whole-cell patch clamp technique based on the methods previously described [9]. An Axopatch 200B

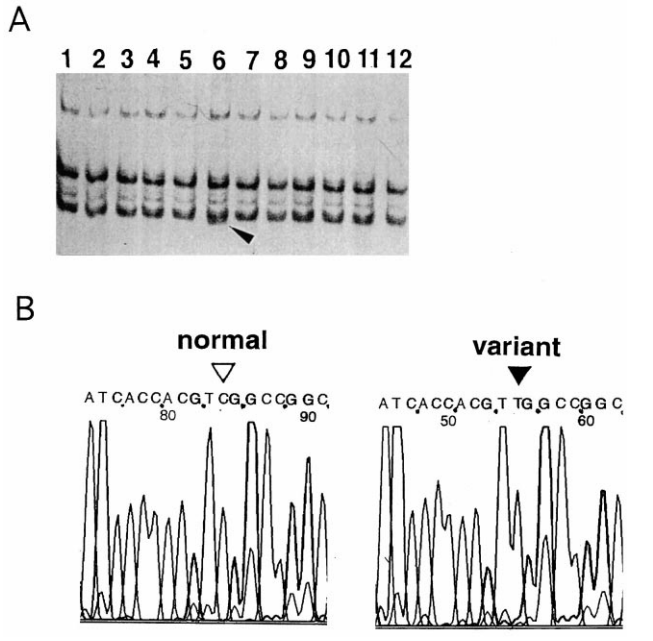

Fig. 2. A: PCR-DCP analysis of exon 28. Genomic DNAs from IVF patients were amplified by the PCR for exon 28 and the PCR products were electrophoresed in an 8% polyacrylamide gel after heat denaturation. Lanes 1–5: patients with Brugada syndrome, lanes 6–8: IVF patients without Brugada syndrome-type ECG, lanes 9–12: healthy controls. An abnormal DCP fragment shown by an arrow was found in the patient VF06 (lane 6). B: DNA sequencing data of *SCN5A* clones from VF06. Sequences of clones representing normal (left) and abnormal DCP patterns (right) are shown. The mutant clone had a C to T transition.

patch clamp amplifier was used to measure whole-cell currents with series resistance compensation (Axon Instruments). The holding potential for all pulse protocols was −150 mV. Pipette resistance was 1.0–2.5 MΩ. The bath solution contained 36 mM NaCl, 109 mM *N*-methyl glucamine, 4 mM KCl, 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2$, 10 mM HEPES, and 10 mM glucose, pH 7.35. The pipette solution contained 10 mM NaF, 110 mM CsF, 20 mM CsCl, 10 mM EGTA, and 10 mM HEPES, pH 7.35. Voltage-clamp command pulses were generated using pCLAMP6 (Axon Instruments) and currents were filtered at 5 kHz (−3 dB, 4-pole Bessel filter). Experiments were done at room temperature (20–22°C) unless otherwise stated. The data were analyzed using Clampfit (Axon Instruments) and SigmaPlot (SPSS Science). Results are presented as means ± S.E.M. and the statistical comparisons were made using the unpaired Student's *t*-test to evaluate the significance of the difference between means. Statistical significance was assumed for $P < 0.05$.

3. Results

3.1. Molecular genetics

PCR-DCP analysis showed an aberrant band in exon 28 of an IVF patient (VF06, Fig. 2A). This pattern was not observed for 300 normal chromosomes, consistent with a disease-related mutation. DNA sequencing confirmed a C to T transition leading to amino acid substitution of leucine for serine 1710 (S1710L; Fig. 2B), located at the pore-forming loop (P-loop) between segments 5 and 6 of domain 4. Amino acid sequence alignment shows that the P-loop of D4 is highly conserved among different Na^+ channel isoforms and the residues corresponding to the S1710 of *SCN5A* are identical among them (Fig. 3). The heterozygous state of this patient was confirmed by direct sequencing of the PCR products from genomic DNA (data not shown). No other *SCN5A* mutations were found in the patient V06. The other 24 IVF patients did not show aberrant bands on DCP analysis for any exons of *SCN5A*.

3.2. Functional analysis of the S1710L mutation

In the absence of further genetic data on this family, we chose to determine whether the mutation affects sodium channel function. This was accomplished by expressing a recombinant human heart sodium channel in a heterologous cell line and recording currents with the whole-cell patch clamp technique. One remarkable biophysical property observed for the macroscopic Na^+ currents of heterologously expressed S1710L channel is the accelerated current decay (Fig. 4A).

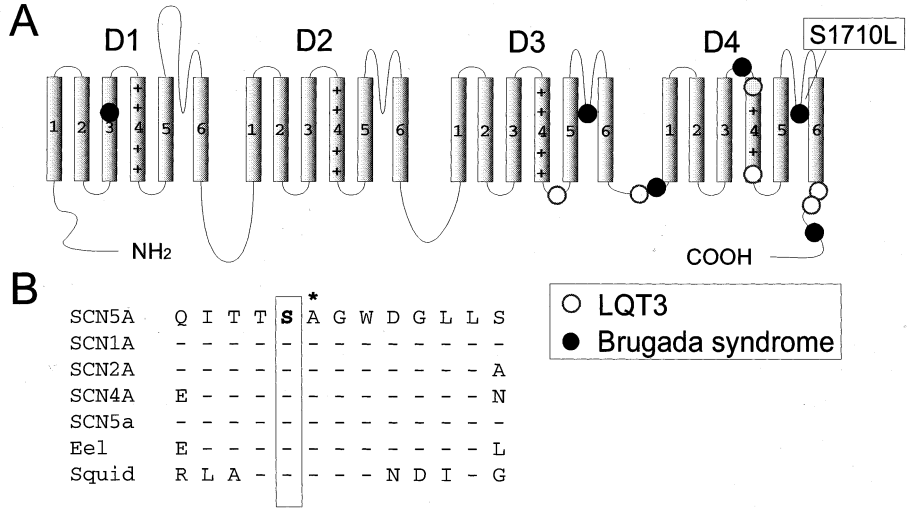

Fig. 3. A: *SCN5A* mutations in inherited cardiac arrhythmia. The predicted topology of cardiac Na^+ channel is illustrated with location of known mutations associated with either LQT3 (○) or Brugada syndrome (●). B: Amino acid sequence alignments of *SCN5A* with related Na^+ channel sequences are shown in the lower panel (SCN5A: human heart [M77235], SCN1A: human brain type I [X65362], SCN2A: human brain type II [M94055], SCN4A: human skeletal muscle [M81758], SCN5a: rat heart [M27902], Eel: eel electroplax [M22252], Squid: squid axon [L19979]; GenBank accession numbers are given in square brackets). The asterisk indicates the alanine located at the P-loop of D4, one of the responsible residues for the ion selectivity filter of voltage–gate Na^+ channels.

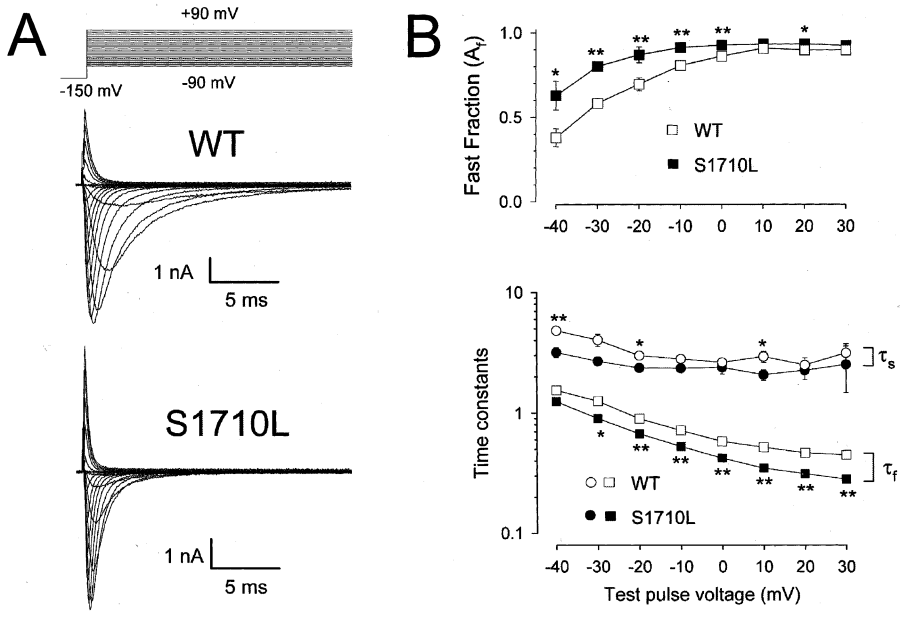

Fig. 4. A: Representative whole-cell current records obtained from HEK-hβ_1 cells transfected with either WT or S1710L Na^+ channels. Currents were recorded from a holding potential of −150 mV stepped from −90 mV to +90 mV during 20 ms in 10 mV increments. Currents were normalized and superimposed to illustrate differences in the time course of current decay. B: The time course of inactivation was fit with a two-exponential function: $I_t/I_{max} = A_\infty + A_f \times \exp(-t/\tau_f) + A_s \times \exp(-t/\tau_s)$, where A_∞ is a constant value, A_f and A_s are the fractions of fast and slow inactivating components, τ_f and τ_s are the time constants of fast and slow inactivating components, respectively. The fraction of fast component (A_f) is shown in the upper panel, and the fast time constants (τ_f, squares) and the slow time constant (τ_s, circles) are illustrated in the lower panel. Statistical significance in the difference between WT (open, $n=10$) and S1710L (solid, $n=9$) are shown (*$P<0.05$, **$P<0.01$).

The time course of inactivation fit with a two-exponential function revealed that the fraction of rapidly inactivating component (A_f) was significantly larger and the time constants of fast components (τ_f) were significantly smaller in S1710L than in WT at test pulse voltages between −40 mV and +30 mV (Fig. 4B). Since it was recently reported that a higher temperature unmasks the abnormalities of channel kinetics in a Brugada syndrome mutant Na^+ channel T1620M [10], we tried patch clamp experiments at 32°C. Macroscopic current decay at 32°C was significantly faster for both WT and S1710L channels than at room temperature, however, S1710L displayed current decay even faster than WT, suggesting that the time course of inactivation of S1710L was significantly faster than WT regardless of the temperature (unpublished observation, Shirai et al.). We did not carry out further experiments under these conditions because capacitance compensation tended to become too difficult to obtain consistent and reliable data as temperature increased. Moreover, comparable functional abnormalities of S1710L were shown by patch clamp studies using native HEK cells without transfection of β_1 subunit and two-electrode voltage clamp experiments using *Xenopus* oocytes (data not shown), suggesting that S1710L does not exhibit phenotypic discrepancies depending on the expression system [11] or kinetic modifications by co-expressed β_1 subunit [12] demonstrated in the T1620M mutant channel.

The voltage dependence of steady-state inactivation of S1710L was significantly shifted towards more negative potential by nearly 25 mV ($V_{1/2}$: WT = −80.8 ± 2.1 mV, $n=9$; S1710L: −105.1 ± 2.4 mV, $n=9$; $P<0.001$) and the slope factors were significantly larger in S1710L (k: WT = 7.7 ± 0.18, S1710L = 8.5 ± 0.24, $P<0.05$) (Fig. 5A). Conversely, the voltage-dependence of activation of S1710L was significantly shifted towards a more positive potential by ~18 mV ($V_{1/2}$: WT = −44.1 ± 0.3 mV, S1710L: −26.4 ± 0.9 mV; $P<0.001$) and the slope factors were significantly larger (k: WT = 7.6 ± 0.23, S1710L = 8.5 ± 0.28; $P<0.05$). Consequently, S1710L channels have severe functional defects resulting in substantially smaller overlap in the relationship between channel inactivation and activation (window current).

The time course of recovery from inactivation was significantly slower in S1710L than in WT (Fig. 5B). The time constant of fast recovery component (τ_f) was significantly larger in S1710L (3.2 ± 0.29 ms, $n=8$) than in WT (1.6 ± 0.17 ms, $n=10$) ($P<0.001$). The time constant of slow recovery component (τ_s; WT = 27.7 ± 4.9 ms, S1710L = 33.3 ± 4.5 ms), and the fractions of fast recovery component (A_f; WT = 0.83 ± 0.02, S1710L = 0.84 ± 0.02) and slow recovery component (A_s; WT = 0.15 ± 0.02, S1710L = 0.16 ± 0.04) were comparable.

4. Discussion

In this study, we have identified a novel *SCN5A* missense mutation S1710L in an IVF patient who lacks documentation of a typical ECG pattern of the Brugada syndrome (coved or saddleback type ST elevations in V_{1-3}). It has been a matter of controversy whether this IVF subgroup is a phenotypic variation of the Brugada syndrome or a disease entity clinically and genetically distinct from the Brugada syndrome, because ECGs of Brugada syndrome patients are profoundly affected by an autonomic nervous system tone [13] and exhibit time-dependent changes with occasional normalization. Genotype–phenotype co-segregation data are not available in this study, however, identification of a novel mutation of *SCN5A* associated with a symptomatic IVF patient who lacks docu-

mentation of a typical ECG of the Brugada syndrome has genetic relevance indicating that Brugada syndrome and this IVF subgroup are at least genetically overlapped, and possibly allelic disorders resulting from defects of *SCN5A* like LQT3 form of congenital long QT syndrome and hereditary A-V block [14].

Biophysical properties of the S1710L mutant Na^+ channels are: (1) accelerated time course of inactivation, (2) hyperpolarizing shift of steady-state inactivation curve, (3) depolarizing shift of activation curve, and (4) delayed recovery from inactivation. Based on these findings, it is expected that the mutant S1710L channels enter the inactivated state from open state with significantly faster rates than WT, and the availability for opening was markedly reduced at membrane potentials negative to the activation threshold (~ -50 mV for normal heart), while the activation threshold itself was significantly elevated in S1710L compared to WT (Fig. 3C). These biophysical properties could potentially result in a decrease of net Na^+ currents at each cardiac cycle, and are compatible with the electrophysiological conduction disturbance clinically

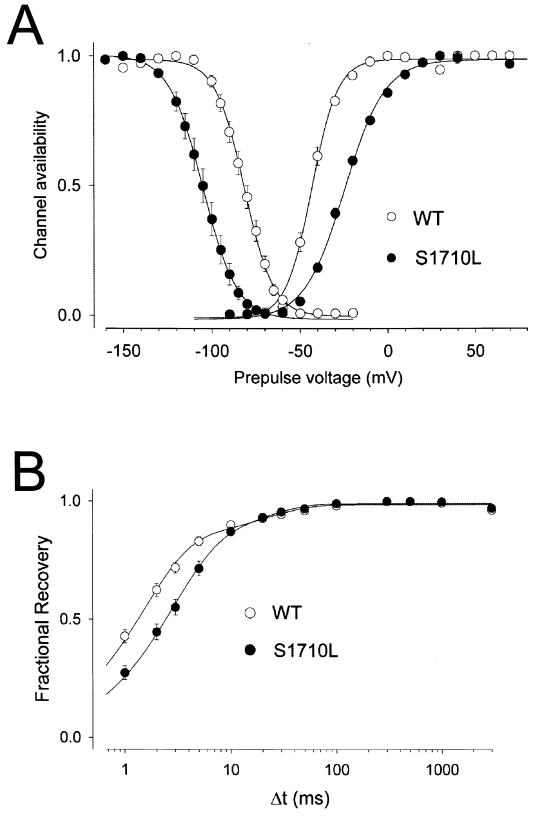

Fig. 5. A: Voltage-dependence of steady-state inactivation and activation of WT and S1710L expressed in HEK-hβ_1 cells. Steady-state inactivation at -20 mV from 100 ms prepulses ranging between -160 mV and -20 mV. Curves were fit with the Boltzmann equation, $I/I_{max} = [1+\exp((V-V_{1/2})/k)]^{-1}$ to determine the membrane potential for half maximal activation ($V_{1/2}$) and the slope factor k. The steady-state inactivation of S1710L was significantly shifted in negative direction, and the activation in positive direction. B: Time course of recovery from inactivation of WT and S1710L were assessed by a double pulse protocol consisting of a 500 ms prepulse to +20 mV designed to fully inactivate all channels, followed by a variable duration interpulse interval (Δt) at -150 mV and a test pulse of -20 mV. The pulse protocol cycle time was 5 s. Recovery from inactivation was analyzed by fitting data to a two-exponential function.

observed for the patient VF06 (H-V prolongation, rate-dependent RBBB and intraventricular conduction disturbance). Furthermore, a decrease in the Na^+ current will cause an outward shift of the current active at the end of phase 1, and a marked abbreviation of action potential in the epicardium but not in the endocardium, because the transient outward K^+ current (I_t) is predominantly expressed in epicardium, which in turn creates a transmural voltage gradient during repolarization and disparity of the refractory period, and provides the substrate reentry [15,16]. However, the lack of ST elevation in ECG of this patient cannot be verified by the above explanation and is to be further delineated.

Enhanced inactivation due to accelerated current decay is not a unique feature specifically observed for S1710L. Similar channel property was observed in Brugada syndrome mutation T1620M, although only at higher temperature, and was regarded as one of the relevant mechanisms for ST elevation [10]. Clinically, however, the patients with a T1620M mutation showed a typical ECG pattern of the Brugada syndrome, while the patient with a S1710L mutation did not. These findings suggest that an accelerated time course of inactivation per se may not be the primary mechanism of the ECG signature of Brugada syndrome.

Brugada syndrome and the LQT3 form of congenital long QT syndrome are allelic disorders resulting from the defects of *SCN5A*. Persistent Na^+ currents due to dysfunctional channel inactivation are commonly observed for most LQT3 mutant channels and regarded as the molecular basis of QT prolongation in LQT3 [17]. In contrast, the molecular mechanisms underlying Brugada syndrome and other IVF appear more complicated, because heterologously expressed mutant Na^+ channels reported so far exhibit a wide variety of functional properties; for instance, T1620M mutation gives rise to a depolarizing shift of the steady-state inactivation curve [3,12] with an accelerated current decay only at higher temperatures [10], while the R1512W mutant showed a hyperpolarizing shift of steady-state activation and inactivation, and A1924T caused a negative shift of the activation curve alone [18]. Moreover, an in-frame stop codon in the D4/S6 region results in a non-functional channel [3]. Furthermore, it is still obscure why the dysfunctional heterologously expressed S1710L channels appear more severe than most other Brugada syndrome mutations while the ECG abnormalities of S1710L apparently seem more subtle than those of the Brugada syndrome.

The residue S1710 of hH1 is located at the P-loop between S5 and S6 of domain 4 and the corresponding residue is highly conserved among different Na^+ channel isoforms (Fig. 3). Recent structure–function studies have revealed that S1710 is located at the deepest region of the P-loop. It is believed that the ion selectivity of the Na^+ channel is determined by a ring of amino acids of P-loops of all four domains [19,20] and the major determinants are the residues so-called 'DEKA' (D372, E898, K1419, and A1711 of the hH1 sequence). Since S1710 is adjacent to the residue A1711, it is speculated that the mutant Na^+ channel S1710L may have additional functional alterations such as in ion permeation properties. These issues are currently being investigated.

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