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Original Article

A novel mutation in *SCN5A*, delQKP 1507–1509, causing long QT syndrome: Role of Q1507 residue in sodium channel inactivation

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Abstract

Inherited long QT syndrome (LQTS) is caused by mutations in six genes including *SCN5A*, encoding the α-subunit of the human cardiac voltage-dependent sodium channel $hNa_v1.5$. In LQT3, various mutations in *SCN5A* were identified, which produce a gain of channel function. The aim of this study was to screen *SCN5A* for mutations in a family with the LQT3 phenotype and to analyze the consequences of the mutation on the channel function. By polymerase chain reaction-denaturating high performance liquid chromatography-sequencing, we identified a novel deletion in *SCN5A*, delQKP 1507–1509, in the DIII–DIV linker of the sodium channel. The $hNa_v1.5$/delQKP1507–1509, $hNa_v1.5$/delQ1507 and $hNa_v1.5$/Q1507A mutants were constructed in vitro, mutant channels were expressed in the tsA201 human cell line and studied using the whole-cell configuration of the patch clamp technique. A persistent inward sodium current of 1–1.5% of maximum currents measured at −30 mV in all mutant sodium channels was recorded, which was nearly completely blocked by the sodium-channel blockers tetrodotoxin and lidocaine. The deletion mutants resulted in a significant shift of steady-state activation to more depolarized voltages. The delQ1507 showed a small shift of steady-state inactivation towards more negative potentials, whereas no significant shifts were observed in both steady-state activation and inactivation in Q1507A compared to the wild-type $Na_v1.5$ sodium channels. The novel *SCN5A* mutation, delQKP, induces a residual current as previously shown for other *SCN5A* mutations causing LQTS. DelQKP shares the deletion of Q1507 with the formerly known delKPQ 1505–1507. Our data suggest that Q1507 plays an important role in fast sodium channel inactivation.

Keywords: Arrhythmia; Long QT syndrome; Sudden death; *SCN5A* gene; Sodium channel; Persistent inward sodium current

1. Introduction

The congenital long QT syndrome (LQTS) is an inherited cardiac disease characterized by alteration of ventricular repolarization with malignant ventricular arrhythmias leading to syncope, torsades de pointes and sudden death [1–3]. The autosomal dominant Romano–Ward syndrome is caused by mutations in six different genes most of them encode ion channels or their regulatory subunits [4,5]. The *SCN5A* gene encodes the α-subunit of the human cardiac voltage-dependent sodium channel $hNa_v1.5$, which activates the production of a depolarizing inward sodium current, I_{Na}, with prompt inactivation within milliseconds [6]. Mutations in *SCN5A* leading to LQT3 are in general associated with a gain of channel function with increased late sodium current leading to a delayed repolarization and sustained action potential duration. The first and the most extensively studied mutation in LQT3 is the deletion of three amino acids KPQ at positions 1505–1507 in *SCN5A* [7,8]. The mutation is localized in the linker between the domains III and IV of $Na_v1.5$ sodium channel proteins. In fact this region forms the inactivation

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gate of the channel [9]. Biophysical studies of delKPQ have elucidated its various effects on sodium channel activation and inactivation [10,11].

The aim of this study was to screen *SCN5A* gene for a mutation in a family with a typical LQT3 phenotype and to analyze the consequences of the mutation on the sodium channel function. We identified a novel mutation in *SCN5A*, delQKP, which deletes the three amino acid residues Gln1507–Lys1508–Pro1509 in the DIII–DIV linker region of the sodium channel. DelQKP 1507–1509 shares the deletion of the glutamine residue at position 1507 (Gln1507) with the previously known mutation delKPQ 1505–1507 [12]. Using the patch clamp technique we characterized delQKP, delQ1507 and Q1507A mutations in $hNa_v1.5$ expressed in mammalian cells and showed that the Q1507 residue plays an important role in fast channel inactivation.

2. Methods

2.1. Clinical evaluation

Subjects II-2 (index patient) and III-4 underwent clinical and cardiovascular examination including medical history, 12-lead electrocardiogram (ECG), 24-h ECG recording (subject II-2) and transthoracic echocardiography. In the other family members participating in the study, 12-lead ECG was performed and medical history was obtained. The QT interval was corrected for heart rate using Bazett's formula.

2.2. Molecular genetics

Informed consent was obtained from all participating subjects. The investigation conforms with the principles outlined in the Declaration of Helsinki [13]. Genomic DNA was extracted from blood lymphocytes. In subject II-2, exons 1 through 28 of *SCN5A* were amplified by polymerase chain reaction (PCR), using primers designed in intronic flanking sequences, according to the gene sequences [14]. Denaturating high performance liquid chromatography (DHPLC) was performed on DNA-amplification products of all exons at two temperature conditions [15]. Abnormal DHPLC profiles were analyzed by sequence reaction in both strands of the exon, using big dye termination mix. The sequencing products were analyzed by cycle sequencing on an automated laser fluorescent DNA sequencer (ABI prism 3100, Applied Biosystems, Foster City, CA, USA). In subjects I-1, II-1, III-1, III-2, III-3, III-4 and III-5, exon 26 of *SCN5A* was amplified and sequencing reaction in both strands was performed as described before above. The novel deletion QKP was absent in 200 normal alleles.

2.3. Mutagenesis

Mutant sodium channels delQKP, delQ1507 and Q1507A were generated using QuickChange™ site-directed mutagenesis kit, according to the manufacturer's instructions (Stratagene, La Jolla, CA, USA). The $hNa_v1.5$/mutants were constructed using the following mutagenic sense and antisense primers:

5′-GGC TCC AAG AAG CCC–ATC CCA CGG CCC C-3′ and
5′-CAG GGG CCG TGG GAT GGG CTT–CTT GGA GCC-3′
for $hNa_v1.5$/delQKP1507–1509
5′-GGC TCC AAG AAG CCC–AAG CCC ATC CCA CGG-3′ and
5′-CCG TGG GAT GGG CTT–GGG CTT CTT GGA GCC-3′
for $hNa_v1.5$/delQ1507
5′-G GGC TCC AAG AAG CCC GCG AAG CCC ATC CCA CGG C-3′ and
5′-G CCG TGG GAT GGG CTT CGC GGG CTT CTT GGA GCC C-3′
for $hNa_v1.5$/Q1507A.

The presence of the mutation was confirmed using automatic sequencing. Deletion mutant and the wild-type (WT) $hNa_v1.5$ in pcDNA1 constructs were purified using Quiagen columns (Quiagen Inc., Chatsworth, CA, USA).

2.4. Transfections of tsA201 cell line

The tsA201 cell line, a mammalian cell line, is derived from human embryonic kidney HEK293 cells by stable transfection with simian virus 40 (SV40) large T antigen [16]. Cells were grown in high glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, L-glutamine (2 mM), penicillin G (100 U/ml) and streptomycin (10 mg/ml) (Gibco BRL Life technologies, Burlington, Ont., Canada) and incubated in a 5% CO_2 humid atmosphere incubator. The tsA201 cells grown to 40–50% confluence on 100-mm plates were transfected with WT or mutant $hNa_v1.5$ cDNAs (5 μg) along with human β_1-subunit and pCD8-IRES-β_1 (5 μg) using the calcium–phosphate method. The human β_1-subunit and CD8 were constructed in pIRES bicistronic vector (pCD8-IRES-β_1). Using this strategy, transfected cells that bind beads will also express the β_1-subunit protein. For patch clamp experiments, 2–3 days post-transfection cells were incubated for 2 min in a medium containing anti-CD8-a coated beads (Dynabeads M-450 CD8-a) [17]. The unattached beads were removed by washing with extracellular solution. Beads were prepared according to the manufacturer's instructions (Dynal A.S., Oslo, Norway). Cells expressing CD8-a on their surface fixed the beads and were distinguished from non-transfected cells by light microscopy.

2.5. Patch clamp method

Macroscopic sodium currents from transfected cells were recorded using the whole-cell configuration of the patch clamp technique [18]. Patch electrodes were made from 8161 Corning borosilicate glass and coated with Sylgard (Dow-Corning, Midland, MI, USA) to minimize their ca-

pacitance. Patch clamp recordings were accomplished using low resistance electrodes (<1 MΩ), and a routine series resistance compensation of an Axopatch 200 amplifier (Axon Instruments, Foster City, CA, USA) was performed to values >80% to minimize voltage-clamp errors. To ensure the quality of the voltage clamp, we measured the time constant of membrane charge capacitance ($\tau = R_{series} * C_m$) $\tau = 4.71 \pm 0.43$ μs, $n = 40$. The C_m and R_{series} were obtained respectively from the capacitance and the series resistance compensation of the Axopatch 200. These values showed high speed for charging cell capacitance and, therefore, demonstrated the satisfactoriness of the voltage clamp. Furthermore, the series resistance was in the range of 0.3 MΩ. For this level of compensation and with 0.3 MΩ of series resistance the voltage error was less than 1 mV for the largest current we recorded in this study. This level of error is acceptable and demonstrates that the voltage clamp is adequate.

Voltage-clamp command pulses were generated by microcomputer using pCLAMP software v8.0 (Axon Instruments, Foster City, CA, USA). Sodium currents were filtered at 5 kHz digitized at 10 kHz and stored on a microcomputer equipped with an AD converter (Digidata 1300, Axon Instruments, Foster City, CA, USA). Experiments were performed 10 min after obtaining the whole-cell configuration to allow the current to stabilize and reduce the time-dependent shift of gating [19].

2.6. Solutions and reagents

For whole-cell recording, the patch pipette contained (mM): 35 NaCl, 105 CsF, 10 EGTA and 10 Cs-HEPES (pH = 7.4). The bath solution contained (mM): 150 NaCl, 2 KCl, 1.5 $CaCl_2$, 1 $MgCl_2$, 10 glucose and 10 Na-HEPES (pH = 7.4). A correction of the liquid junction potential of –7 mV between patch pipette and the bath solutions was made. Tetrodotoxin (TTX) and lidocaine were purchased from Sigma (St. Louis, MO, USA). Experiments were performed at room temperature (22–23 °C).

2.7. Statistical analysis

When indicated, Student *t*-tests were performed using statistical software in SigmaPlot (Jandel Scientific Software, San Rafael, CA, USA). Differences were considered significant at a P value <0.05.

3. Results

The proband (subject II-2) is a 41-year-old woman who was diagnosed with LQT3 during the development of deep vein thrombosis (Fig. 1A). At this occasion she was found to have bradycardia, but was asymptomatic. Between the age of 12 and 16, she had suffered recurrent syncope, which was triggered by fits of anger. The episodes occurred reproducibly and were reportedly short lived and followed by prompt complete recovery. Syncope never recurred after the age of 17. The 12-lead ECG revealed sinus bradycardia with RR interval of 1240 ms, borderline PR (200 ms) and normal QRS (80 ms) intervals. QTc interval was prolonged (576 ms) and T-waves were biphasic with a delayed onset (Fig. 1B). The 24-h ECG recording showed a minimal and mean heart rate of 30 and 59 bpm, respectively. During housekeeping activities, heart rate increased to a maximum of 124 bpm. QT intervals varied between 400 ms at maximal heart rate (QTc 589 ms) and 820 ms at minimal heart rate (QTc 580 ms). Episodes of sinus arrhythmia were present and shorter RR intervals were followed by biphasic T-waves, whereas longer RR intervals were followed by peaked T-waves (Fig. 1C). T-wave morphology was consistent with LQT3 phenotype [20,21]. Structural heart disease was excluded by echocardiography. A dual chamber pacemaker was inserted and therapy with atenolol was initiated.

Family members were screened for LQTS and the 12-year-old asymptomatic son (subject III-4) of the proband was diagnosed with LQT3. The 12-lead ECG showed sinus bradycardia (RR interval 1250 ms) and prolonged QTc interval of 595 ms with a biphasic T-wave (Fig. 1B). PR and QRS intervals were normal (180 and 90 ms). Transthoracal echocardiography revealed normal heart structure and function. A single chamber pacemaker was inserted and therapy with metoprolol was initiated. Though he was asymptomatic, he noted an improvement of his exertion capacity after pacemaker insertion.

The 12-lead ECG's of the subjects II-1, II-3, III-1, III-2, III-3 and III-5 revealed normal QTc intervals. There was no history of syncope, sudden death or cardiovascular events in the family.

3.1. Identification of a novel mutation delQKP 1507–1509 in SCN5A

By a PCR-DHPLC approach, an abnormal elution profile was found at 62 and 63 °C in the amplified product of exon 26 in subject II-2 (p) compared to the control (c) (Fig. 2A). Heterozygote DNA sequencing confirmed a deletion of nine base pairs (CAGAAGCCC), corresponding to the three amino acid residues Gln1507–Lys1508–Pro1509 (QKP), in subject II-2 (p) (Fig. 2B). The inframe-deletion disrupts the coding sequence of QKP at the terminal part of exon 26 and is localized in the linker region between DIII–DIV of the $Na_v1.5$ sodium channel. The delQKP mutation was confirmed in subject III-4 and excluded in subjects I-1, II-1, III-1, III-2, III-3 and III-5.

3.2. Persisting I_{Na} in cells transfected with mutant channels

Macroscopic sodium currents were recorded from tsA201 cells expressing WT ($hNa_v1.5$/WT), deletion and substitution mutant channels ($hNa_v1.5$/delQKP, delQ and Q1507A), co-transfected with the human β_1-subunit (Fig. 3). The left panels of Fig. 3 show current traces of WT and mutant

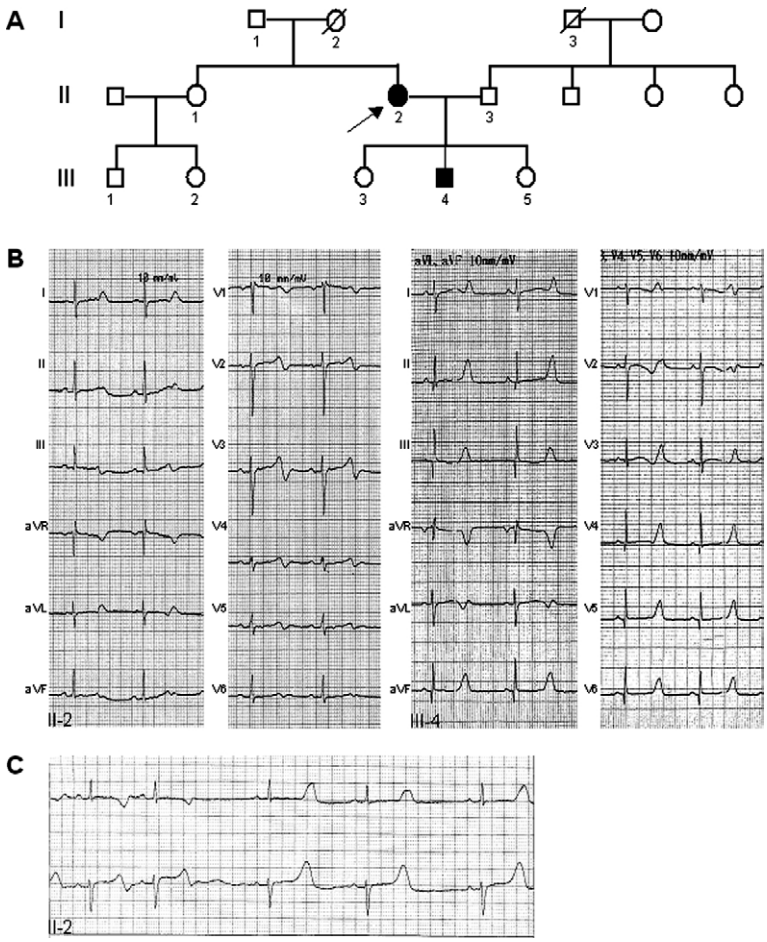

Fig. 1. Subjects II-2 and III-4 with the LQT3 phenotype (A) (black circle and square, proband indicated by arrow). The 12-lead ECG of subject II-2 with QTc interval of 576 ms and delayed T-wave onset with biphasic T-wave morphology and of subject III-4 with QTc interval of 595 ms with a delayed onset biphasic T-wave (B) (ECG tracings recorded at 25 mm/s). Rhythm strip from the 24-h ECG recording of subject II-2 showing variable T-wave morphology dependent on preceding RR interval (C).

sodium channels, and right panels the corresponding current–voltage (*I/V*) relationship. The delQKP mutant sodium channels resulted in sodium currents exhibiting fast activation and inactivation kinetics and, however, exhibiting a persistent I_{Na} of 2.1 ± 0.9% of sodium currents measured at 100 ms in response of a test pulse of –30 mV (Fig. 3A,B). For WT channels sodium currents activated at –80 mV and reached a peak at –40 mV; however in mutant sodium channels a significant shift of the *I/V* relationship was observed with current activating at –70 mV and reaching a peak at –30 mV. Since the delQKP mutation shares the deletion of Q1507 with the previously studied delKPQ1505–1507 in the DIII–DIV linker [12], we investigated whether the deletion of the glutamine residue (delQ1507) and its substitution by an alanine (Q1507A) exhibited similar persistent I_{Na}. Sodium currents were also measured from delQ1507 to Q1507A mutant channels. Similar residual currents of 1.6 ± 0.1% and 1.1 ± 0.5% were observed with delQ1507 and Q1507A, respectively (Fig. 3C,D). A similar shift of the *I/V* relationship was observed with delQ1507 mutant and no significant shift was observed with the Q1507A mutant. The voltage dependence of inactivation for WT and mutant sodium channels was also assessed by plotting τ_h values vs. voltage and exhibited smaller values over a wild voltage range (Fig. 4).

Steady-state activation and inactivation curves were studied (Fig. 5A). No significant shift of steady-state inactivation curve was observed with delQKP channels (Table 1); the slope factor was not affected. However, steady-state activation curve showed a 12 mV shift towards more positive potentials (Fig. 5A and Table 1). The slope factor of steady-state activation curves was also significantly affected (Table 1). Similar shift of steady-state activation was observed in delQ1507 mutant with a significant effect on slope factor and a slight shift of the inactivation curve to more negative potentials. Mutant Q1507A did not show any significant shifts of steady-state gating compared to the WT channels (Table 1).

The recovery from fast inactivation curves were constructed from data obtained using a double pulse protocol with various interpulse intervals to allow recovery from fast inactivation. The fraction of sodium currents recovered from inactivation during the interpulse were fitted by a single exponential function. Fig. 5B shows the recovery from fast

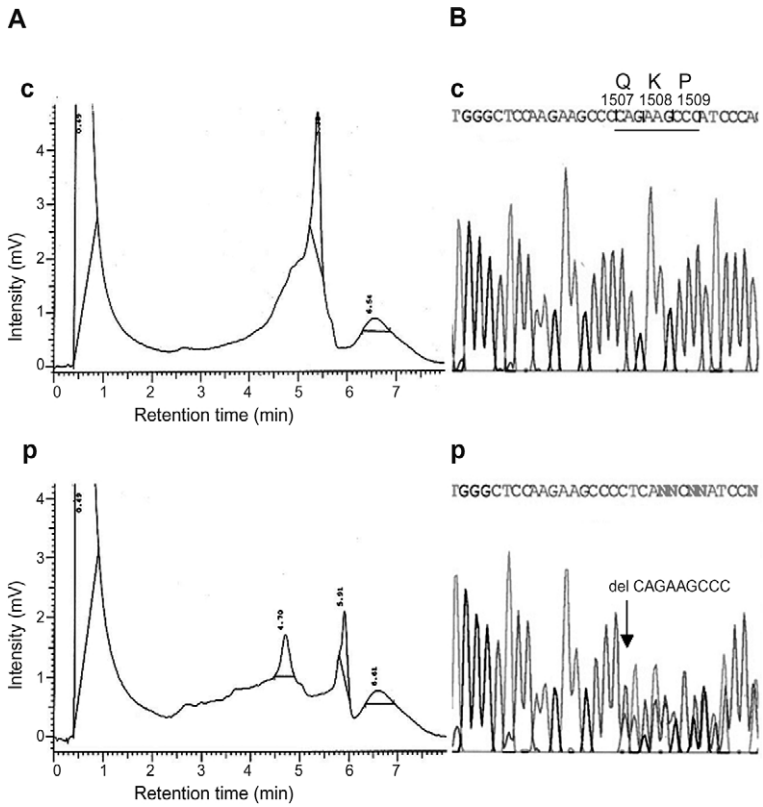

Fig. 2. DHPLC analysis at 62 °C of PCR products of exon 26 *SCN5A*. An abnormal elution profile was observed in the patient (p) compared to the control (c) (A). Automated sequencing identified a heterozygous deletion of nine bases in exon 26 resulting in a deletion of three amino acids QKP Gln1507–Lys1508–Pro1509 in the patient (p) compared to the control (c) (B).

inactivation of delQKP, delQ1507 and Q1507A compared to the WT sodium channel at a holding potential of –120 mV. The time constants of the recovery from inactivation are reported in Table 1 and show a significant reduced time constants of delQKP mutant.

On delQKP mutant channels, the persistent sodium current was reduced to almost zero in the presence of two specific sodium-channel blockers, 10 μM of TTX and 150 μM of the antiarrhythmic drug, lidocaine (Fig. 6A,B). Lidocaine resulted in a 60.32 ± 0.42% reduction in delQKP peak sodium currents. Lidocaine was also capable of reducing the amplitude of the residual current on both delQ1507 and Q1507A mutant sodium channels (Fig. 7A,B).

4. Discussion

The data presented in this paper confirm that the presence of the residual current due to the novel *SCN5A* mutation ($hNa_v1.5$/delQKP) causes LQTS. This is consistent with previous studies indicating that mutations in different regions of *SCN5A* gene are related to the presence of a residual current [10,22–25]. The characterization of distinct human cardiac sodium channel mutations responsible for the LQTS, delKPQ, N1325S and R1644H, showed a persistent I_{Na} [10]. When these mutations were expressed in *Xenopus* oocytes, they each produced a late phase resistance to inactivation, although the mechanisms were different [22]. In N1325S and R1644H, dispersed reopening after the first transient was observed. In contrast, delKPQ showed additional long-lasting bursts resulting in a severe gating defect and therefore the greatest delay in ventricular repolarization. The studies of other *SCN5A* mutations have provided additional insights into the heterogeneity of the disease mechanism. The E1295K mutation is characterized by sustained channel activity only over a very narrow window of voltages compared to WT channels [26], whereas the rate of recovery from inactivation and channel availability are both increased in the I1768V mutation [27]. The R1623Q mutation increases the probability of long openings and produces early reopenings [27,28]. Not a persistent I_{Na}, but a severe destabilization of the inactivated state is the postulated mechanism in the I1768V mutation, most probably due to the faster recovery from inactivation [27,29,30].

Fast and slow modes regulate the gating properties of voltage-gated sodium channel. Although rare, the slow mode of gating characterizes the activity of sodium channel [31]. This slow mode of gating is exacerbated in KPQ deletion mutant [11]. Similarly, the presence of a persistent sodium current in QKP deletion mutant could result from an increased shift from fast to slow mode of gating of sodium channel. Further single channel recording is necessary to

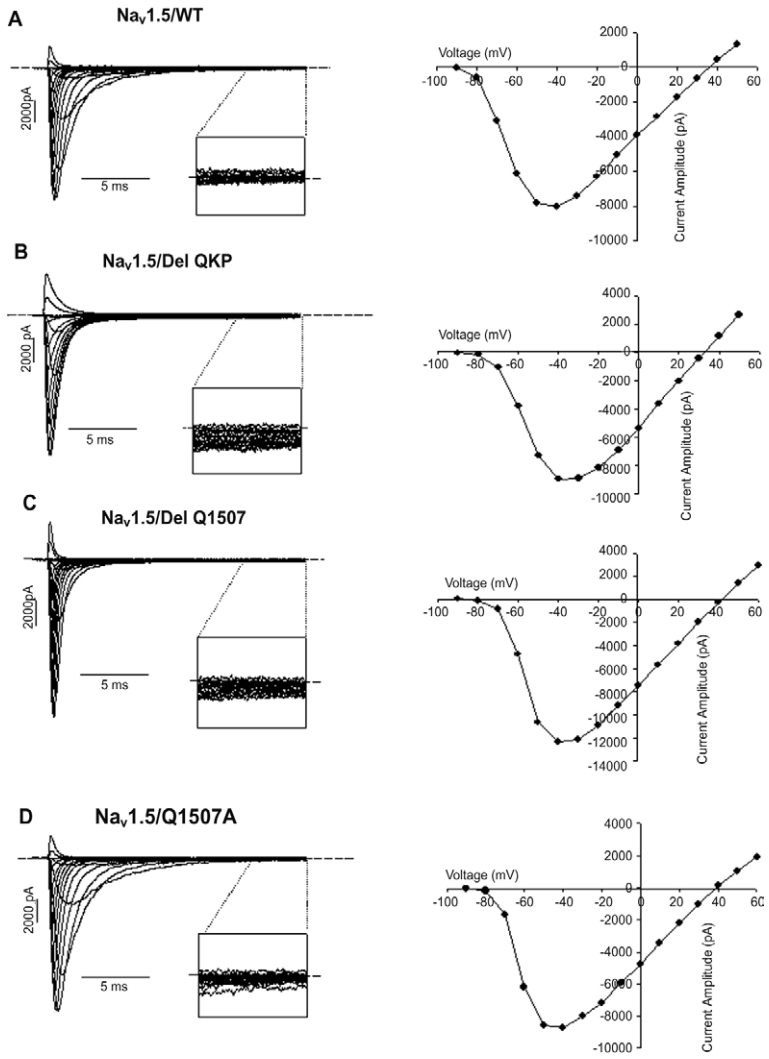

Fig. 3. Left panels represent whole-cell current traces recorded from a tsA201 cell expressing hNa$_v$1.5/WT (A), hNa$_v$1.5/DelQKP (B), hNa$_v$1.5/DelQ (C) and hNa$_v$1.5/Q1507A (D). Insets are enlarging currents to highlight the persistent sodium currents. Sodium currents were elicited from a holding potential of –140 mV to a test potential starting from –90 to +60 mV, in 10 mV increments. The dotted lines represent the zero current. Mutant and WT hNa$_v$1.5 sodium channels were co-expressed by transient transfection with the β_1-subunit. The right panels represent the *I/V* relationship of the corresponding current traces of the left panels.

verify this hypothesis. This mechanism of action is corroborated by the block of the persistent sodium current by sodium-channel blockers, such as TTX and the class I anti-arrhythmic drug, lidocaine.

Interestingly, the novel delQKP is localized in the same channel linker region as delKPQ [12], and shares the deletion of the 1507 residue. We showed in this study that the deletion of Q alone is sufficient to induce a residual current with similar shifts in steady-state gating properties of Na$_v$1.5 sodium channels (Table 1). This suggests that the Q1507 plays an important role in normal sodium channel inactivation. Moreover, when a conserved charge was substituted by replacing the glutamine residue with an alanine (Q1507A), this resulted in moderate residual current and kinetics that were intermediate between the deletion mutants and WT channels. Furthermore, the steady-state gating properties of Q1507A mutant were similar to the WT channels. This indicates that the nature of the residue at position 1507 in the DIII–IV

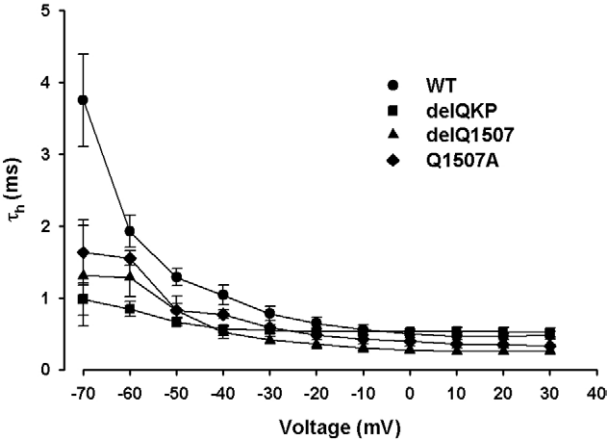

Fig. 4. Voltage dependence of time constants of current decay of sodium currents of WT and mutant sodium channels. The current decay was fitted with two time constants (only the fast component is reported) and the resulted time constants were plotted against the voltage applied. Note the reduction of the time constants over an extended wild voltage range.

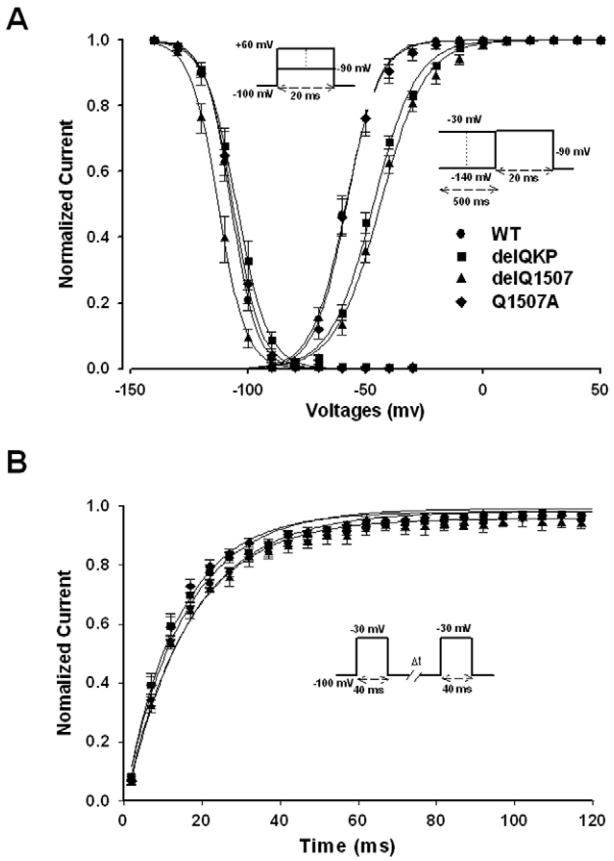

Fig. 5. Voltage dependence of the steady-state activation and steady-state inactivation (h_∞) of $Na_v1.5$/WT (●), $Na_v1.5$/DelQKP (■), $hNa_v1.5$/DelQ (▲) and $hNa_v1.5$/Q1507A (◇) (A). Data points of steady-state activation and inactivation were fitted using a Boltzmann equation with k_v and $V_{1/2}$ representing respectively the slope factor and the half maximal voltage: $I/I_{max} = 1/(1 + \exp((V - V_{1/2})k_v))$, where I_{max} represents the maximum current measured at –140 mV holding potential. The values of $V_{1/2}$ and k_v of steady-state activation and inactivation are reported in Table 1. The recovery from fast inactivation was studied at holding potential of –120 mV using two pulse protocol, with a pre-pulse of 40 ms (B).

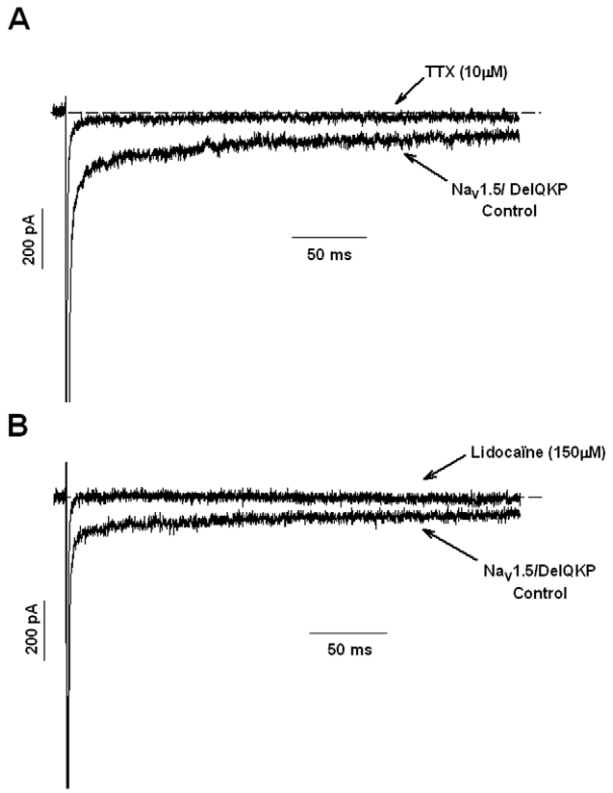

Fig. 6. Effect of 10 μM of TTX on sodium current recorded from cells expressing $Na_v1.5$/DelQKP (A). Effect of 150 μM of lidocaine on sodium current recorded from cells expressing $Na_v1.5$/DelQKP (B). Sodium currents from both panels were recorded from holding potential = –140 mV to a voltage test of –30 mV, before and at steady-state TTX and lidocaine effect. The dashed line represents the zero current.

linker region of $Na_v1.5$ sodium channels is important for channel fast inactivation. A deletion of a lysine residue at position 1500 was recently reported to cause LQTS [32]. DelK1500 resulted also in a shift of activation by 10 mV, we saw a 12 mV shift. These authors raised the possibility that a deletion of a charged residue could underlay an interaction of the inactivation gate and the voltage sensor (S4) of the channel. However, the mechanism underlying the link between activation and inactivation is still unclear. The possibility of a physical link between activation and inactivation is debated [33]. While in this study it was hypothesized that the physical link between activation and inactivation is not necessary for coupling activation to inactivation our data suggest that the physical coupling could take place. In addition to the DII-I–IV linker, a recently characterized mutation implies a role of another linker region, DI–II, in channel inactivation [10,34,35].

On the three mutant sodium channels only delQKP showed significant faster recovery from inactivation. Similar observations were made with other LQT3 mutations [10,34,35]. This suggests a destabilization of the inactivated state of the channel.

Table 1
$V_{1/2}$ and k_v values of steady-state activation and inactivation and the time constant of recovery from inactivation (τ_{rec}) at –120 mV of WT and mutant sodium channels

	Steady-state activation		Steady-state inactivation		Recovery from inactivation
	$V_{1/2}$	k_v	$V_{1/2}$	k_v	τ_{rec}
WT ($n = 11$)	–58.40 ± 1.62	–6.45 ± 0.25	–107.00 ± 0.88	5.16 ± 0.18	17.59 ± 0.90
DelQKP ($n = 11$)	–46.80 ± 0.99*	–8.81 ± 0.34*	–105.00 ± 1.68	5.71 ± 0.15	14.05 ± 1.24*
DelQ1507 ($n = 8$)	–43.70 ± 1.06*	–9.50 ± 0.90*	–113.00 ± 1.54*	5.17 ± 0.26	17.3 ± 0.94
Q1507A ($n = 9$)	–57.70 ± 1.98	–5.46 ± 0.77	–106.00 ± 1.87	4.90 ± 0.13	15.8 ± 1.39

n represents the number of cells tested. Data are expressed as mean ± standard error of the mean (SEM). Statistically significantly differences ($P < 0.05$) are indicated by an asterisk (*).

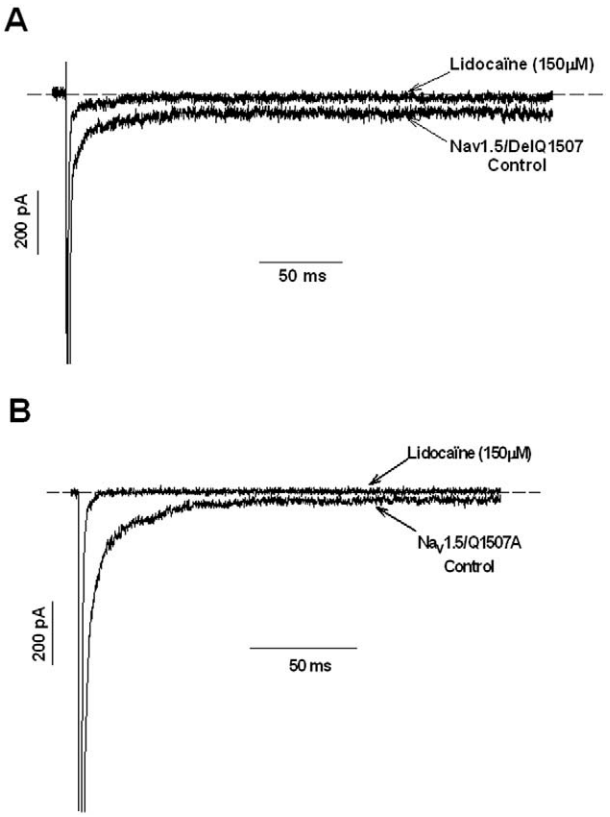

Fig. 7. Effect of 150 µM of lidocaine on sodium currents recorded from cell expressing Na$_v$1.5/DelQ (A) and from cell expressing Na$_v$1.5/Q1507A (B). Sodium currents from both panels were recorded from holding potential = −140 mV to a voltage test of −30 mV, before and at steady-state lidocaine effect. The dashed line represents the zero current.

DelQKP mutation carriers showed certain characteristics that have been associated with other *SCN5A*-linked forms of congenital LQTS, including bradycardia, markedly prolonged QT interval with a long ST segment and a narrow peaked or biphasic T-wave [20,21]. Subject II-2 had marked variability of T-wave morphology and suffered from recurrent short-lived syncope triggered by fits of anger. This is a condition in which sympathetic tone is likely to be elevated, while cardiac events are generally associated with sleep or rest in LQT3 carriers [36]. In this case we cannot conclude between syncope unrelated to ventricular arrhythmias or a distinct phenotype of this new LQT3 mutation.

β-blockers, eventually combined with a pacemaker or implantable cardioverter/defibrillator in case of survived sudden death are considered as therapeutic options [36]. Since both affected individuals had marked bradycardia with severe QT prolongation at long cycle lengths, a pacemaker was implanted, allowing the safe use of β-blockers. Although the role of sodium channel in the sinoatrial node is still debated, it was hypothesized that bradycardia could be related to the persistent sodium current in this tissue [37]. Mexiteline has been studied in *in vitro* models of LQTS and affected reversal of action potential prolongation [38]. The effects of pacing and of mexiletine were recently shown in a mouse model bearing the delKPQ mutation [38]. In heterozygous mice, ventricular pacing reduced dispersion of action potential duration, suppressed early after depolarization and prevented polymorphic ventricular tachycardias, whereas mexiletine shortened action potential duration and suppressed, but did not prevent polymorphic ventricular tachycardias. Although sodium-channel blockers were reported to markedly shorten the QT interval in LQT3 individuals [39], the long-term benefits of sodium-channel blockers in this group remain to be tested. Our in vitro study reported a significant reduction of the residual current in presence of 150 µM lidocaine, indicating that class I antiarrhythmic therapy influences the electrophysiological properties of this novel mutation and might be beneficial for mutation carriers. It remains unclear whether lidocaine has an impact on the triggering of arrhythmias by emotional distress.

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