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A novel SCN5A mutation manifests as a malignant form of long QT syndrome with perinatal onset of tachycardia/bradycardia

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Abstract

Objective: Congenital long QT syndrome (LQTS) with in utero onset of the rhythm disturbances is associated with a poor prognosis. In this study we investigated a newborn patient with fetal bradycardia, 2:1 atrioventricular block and ventricular tachycardia soon after birth.

Methods: Mutational analysis and DNA sequencing were conducted in a newborn. The 2:1 atrioventricular block improved to 1:1 conduction only after intravenous lidocaine infusion or a high dose of mexiletine, which also controlled the ventricular tachycardia.

Results: A novel, spontaneous LQTS-3 mutation was identified in the transmembrane segment 6 of domain IV of the $Na_v1.5$ cardiac sodium channel, with a G→A substitution at codon 1763, which changed a valine (GTG) to a methionine (ATG). The proband was heterozygous but the mutation was absent in the parents and the sister. Expression of this mutant channel in tsA201 mammalian cells by site-directed mutagenesis revealed a persistent tetrodotoxin-sensitive but lidocaine-resistant current that was associated with a positive shift of the steady-state inactivation curve, steeper activation curve and faster recovery from inactivation. We also found a similar electrophysiological profile for the neighboring V1764M mutant. But, the other neighboring I1762A mutant had no persistent current and was still associated with a positive shift of inactivation.

Conclusions: These findings suggest that the $Na_v1.5$/V1763M channel dysfunction and possible neighboring mutants contribute to a persistent inward current due to altered inactivation kinetics and clinically congenital LQTS with perinatal onset of arrhythmias that responded to lidocaine and mexiletine.

Keywords: Congenital long QT syndrome; Fetus; Newborn; Atrioventricular block; SCN5A; Nav1.5; Sodium channels

1. Introduction

Patients with congenital long QT syndrome (LQTS) are at risk of sudden death due to torsade de pointes ventricular arrhythmias [1,2]. To date, over 200 mutations have been identified in six separate ion channel genes that code for the Na or K (I_{Kr} or I_{Ks}) channels or their regulatory subunits [1,2]. Recently, a mutation affecting Ankyrin-B, a cytosolic protein, was also reported to cause type 4 LQTS [3]. Previous studies have documented the benefits of chronic β-blocker therapy, left stellate ganglionectomy, pacemaker

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implantation and implantable cardioverter-defibrillator therapy [2]. However, the prognosis of patients with prenatal or neonatal onset remains poor [4–6]. The SCN5A gene codes for the α-subunit of the human cardiac voltage dependent sodium channel known as $Na_v1.5$. While normal Na channels have virtually complete fast inactivation shortly following opening, the SCN5A mutant channels exhibit a resistance to inactivation and cause a persistent cardiac Na current [2,7]. Although this persistent current is only a small fraction of peak excitatory Na channel current, because of the unique high input impedance of the cardiac action potential plateau, it will prolong the action potential. The subsequent delayed repolarization is responsible for a particular type of congenital LQTS, designated LQT3 [1,2]. This form has been associated with a lower rate of cardiac events but a higher rate of lethal events [1]. We describe here a case of LQTS with perinatal onset of fetal bradycardia as well as neonatal AV block and ventricular tachycardia due to a de novo heterozygous mutation in the transmembrane segment 6 of domain IV (IVS6) of $Na_v1.5$. Using the patch clamp technique, we characterized the V1763M mutation in our patient and the neighboring amino acids (V1764M and I1762A) by replacing them with M and A, respectively, in $hNa_v1.5$ expressed in mammalian cells. Our data show that this region plays an important role in the inactivation kinetics of Na currents and the mutations at this region contribute to persistent inward current due to altered inactivation kinetics.

2. Methods

2.1. Patients

The newborn patient was referred to our institution for perinatal bradycardia and ventricular tachycardia. The parents reported no known consanguinity. The patient and the family members underwent clinical evaluations and 12-lead electrocardiograms. The QT interval was measured on the surface electrocardiogram in lead II and corrected for the heart rate using Bazett's formula. The investigation was performed as per the recommendations of the ethics committee of the institution and the principles outlined in the Declaration of Helsinki.

2.2. Mutational analysis

Genomic DNA was isolated from venous EDTA blood of the infant and the family members using standard procedures. Previously published primer pairs were used to amplify all the exons of KVLQT1, HERG and SCN5A from genomic DNA. Selected exons were screened for the presence of nucleotide sequence polymorphisms by single-strand conformation polymorphism. Amplification reactions were carried out using 40 ng of template DNA, 8 pmol of primers, 2 μl of dNTPs (2.5 mM), 0.8 μl of 25 mM Mg^{2+} and *Taq* polymerase. The PCR products were then analyzed on 12.5% nondenaturating polyacrylamide gels run at 5 and 15 °C as described in the GeneExcel protocol (Pharmacia Biotech). Mutations were detected by differences in migration patterns compared with the wild-type. When abnormal patterns were observed, PCR products were reamplified and sequenced by the dideoxynucleotide chain termination method (DNA Sequencing Kit-Big Dye Terminator Cycle Sequencing v 2.0, PE Biosystems) with fluorescent dideoxynucleotides using an ABI-Prism 373 DNA sequencer (Applied Biosystems). The results were analyzed with the Genotyper program (PE Biosystems).

2.3. Mutagenesis

Mutant $Na_v1.5$/V1763M, $Na_v1.5$/V1764M and $Na_v1.5$/V1762M were generated using a QuickChange TM 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′-CTCCTTCCTCATCGTGATGAACATGTACATTGCC-3′ and 5′-GGCAATGTACATGTTCATCACGATGAGGAAGGAG-3′ for Nav1.5/V1764M 5′-CATCTCCTTCCTCATCATGGTCAACATGTACATTG-3′ and 5′-CAATGTACATGTTGACCATGATGAGGAAGGAGATG-3′ for $Na_v1.5$/V1763M 5′-CATCATCTCCTTCCTCGCCGTGGTCAACATGTAC-3′ and 5′-TACATGTTGACCACGGCGAGGAAGGAGATGATG-3′ for $Na_v1.5$/I1762A (mutated sites are underlined).

Mutant and wild-type $Na_v1.5$ pcDNA1 constructs were purified using Qiagen columns (Qiagen, Chatsworth, CA, USA).

2.4. Transfection of the tsA201 cell line

TsA201 is a mammalian cell line derived from human embryonic kidney HEK 293 cells by stable transfection with SV40 large T antigen [8]. The tsA201 cells were grown in high glucose DMEM supplemented with FBS (10%), L-glutamine (2 mM), penicillin (100 U/ml) and streptomycin (10 mg/ml) (Gibco BRL Life Technologies, Burlington, ON, Canada) and incubated in a 5% CO_2 humidified atmosphere. The cells were transfected using the calcium phosphate method [8] with the following modification to facilitate the identification of individual transfected cells: a cotransfection with an expression plasmid for a lymphocyte surface antigen (CD8-a) was performed [9]. The human sodium channel β_1 subunit and CD8 were constructed in the piRES vector (piERS/CD8/β_1). Using this strategy, transfected cells that bind beads would also express the β_1-subunit. cDNA (5 μg) coding for WT or mutant Na channels and 5 μg of piERS/CD8/β_1 were used. For patch clamp experiments, 2 to 3 day post-transfection cells were incubated for 5 min in a medium containing anti-CD8-a coated beads [9] (Dyna-

beads M-450 CD8-a). Unattached beads were removed by washing. The beads were prepared according to the manufacturer's instructions (Dynal, Oslo, Norway). Cells expressing surface CD8-a fixed the beads and were visually distinguishable from nontransfected cells by light microscopy.

2.5. Patch clamp method

Macroscopic Na currents from tsA201 transfected cells were recorded using the whole-cell configuration of the patch clamp technique [10]. Patch electrodes were made from 8161 Corning borosilicate glass and coated with

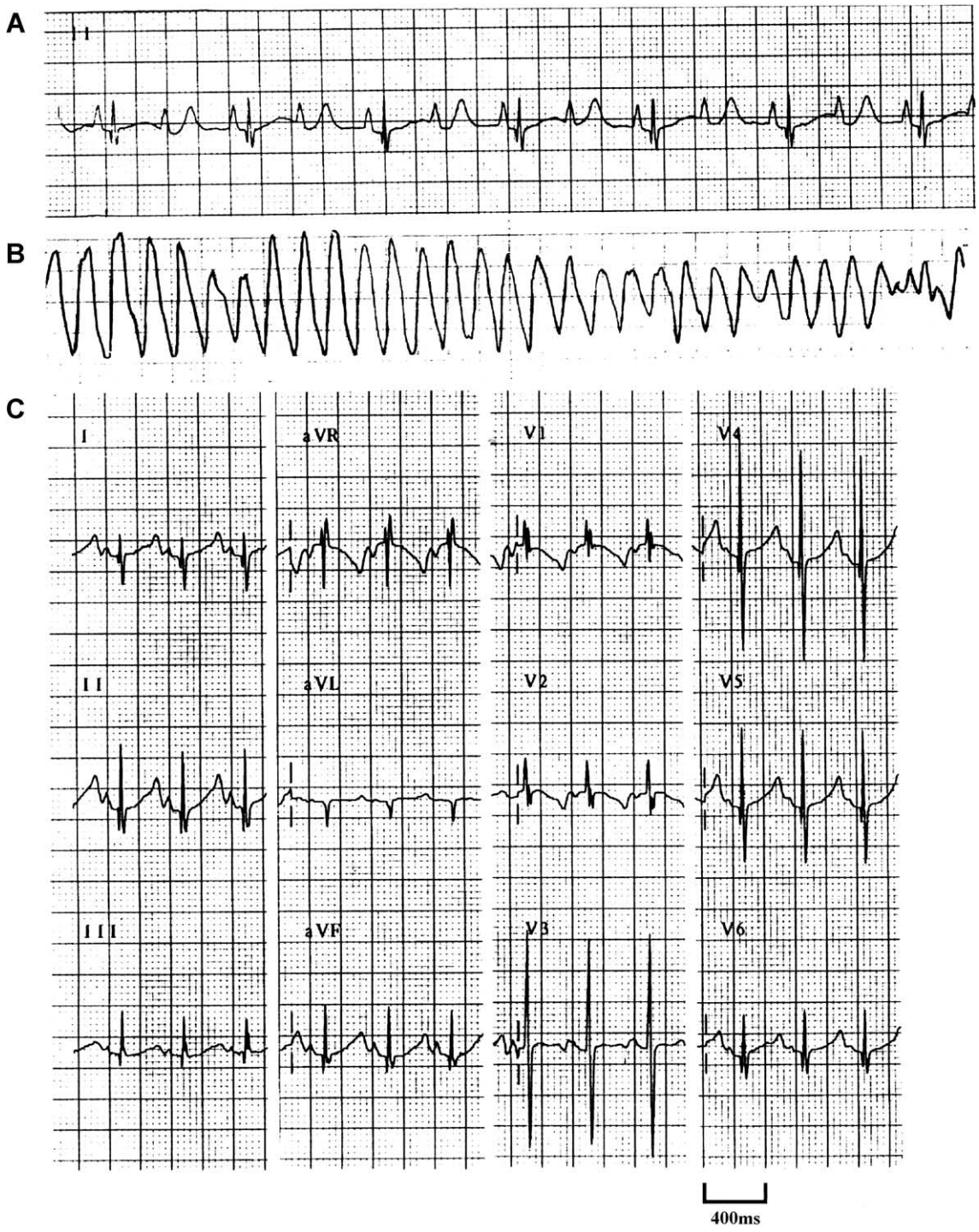

Fig. 1. (A) The rhythm strip shows a prolonged QT interval and 2:1 atrioventricular conduction. (B) The rhythm strip taken during the attack of ventricular tachycardia. (C) Under oral mexiletine of 42 mg/kg/day, the QT interval was shortened and 1:1 AV conduction resumed. Rhythm speed: 25 mm/s.

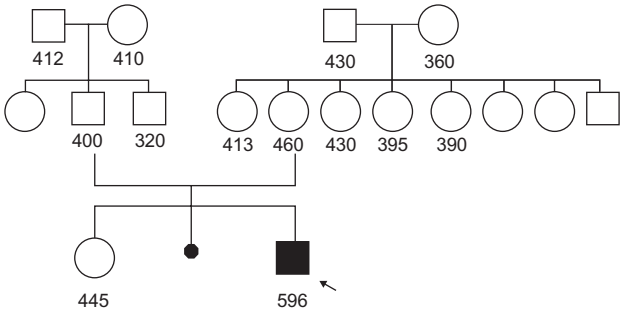

Fig. 2. Pedigree of the study family. The open circles/squares indicate unaffected women/men and the closed circles/squares indicate affected women/men. The small closed circle indicates an unexplained intrauterine death. The arrow indicates the proband.

Sylgard (Dow-Corning, Midland, MI, USA) to minimize their capacitance. Patch clamp recordings were made using low resistance electrodes (<1 MΩ), and a routine series resistance compensation by an Axopatch 200 amplifier (Axon Instruments, Foster City, CA, USA) was performed to values >80% to minimize voltage-clamp errors. Voltage-clamp command pulses were generated by microcomputer using pCLAMP software v8.0 (Axon Instruments). Na currents were filtered at 5 kHz, digitized at 10 kHz and stored on a microcomputer equipped with an AD converter (Digidata 1300, Axon Instruments). Data analysis was performed using a combination of pCLAMP software v9.0 (Axon Instruments), Microsoft Excel and SigmaPlot 2001 for Windows version 7.0 (SPSS, Chicago, IL, USA).

2.6. Solutions and reagents

For whole cell recordings, the patch pipette contained 35 mM NaCl, 105 mM CsF, 10 mM EGTA and 10 mM Cs-HEPES. The pH was adjusted to 7.4 using 1 N CsOH.

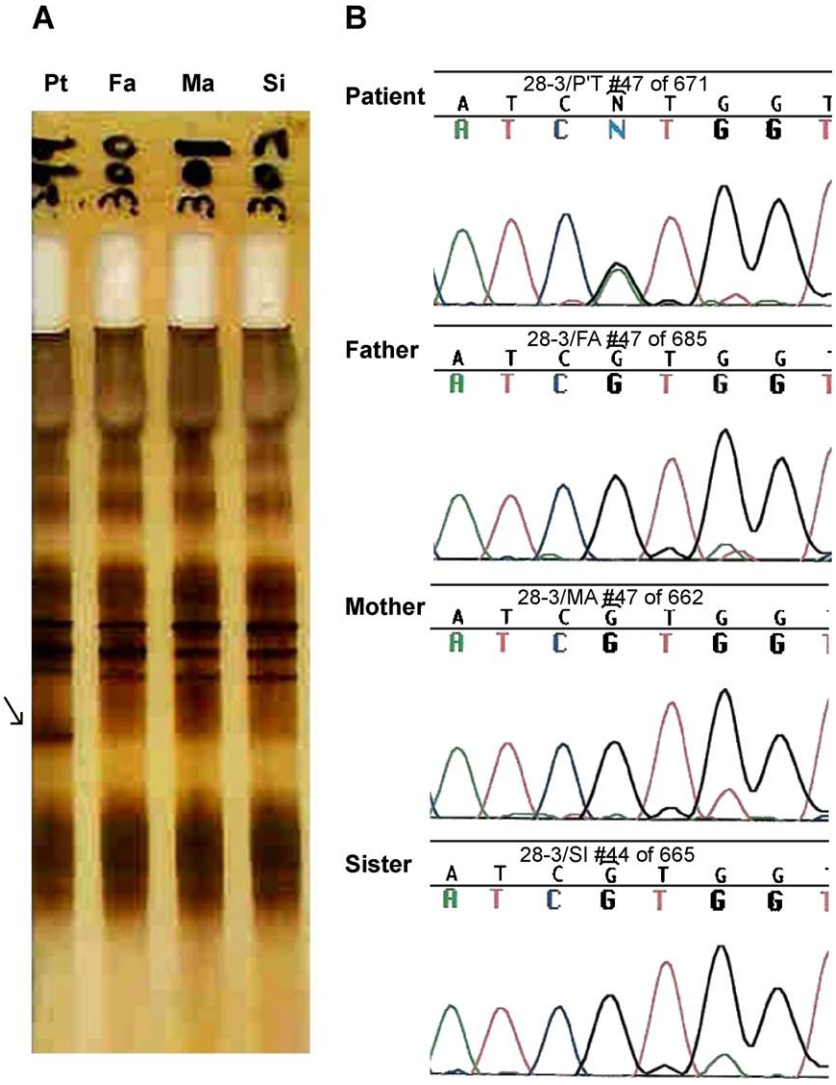

Fig. 3. (A) SSCP analysis of exon 28-3 of the proband and his father, mother and elder sister. Aberrant SSCP conformers (arrow) cosegregate with the disease in the family. (B) DNA sequence chromatograms show a heterozygous mutation with G to A transition resulting in a valine to methionine substitution at codon 1763 in the index patient only. The results from the mother, father and the elder sister were normal.

The bath solution contained 150 mM NaCl, 2 mM KCl, 1.5 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM glucose and 10 mM Na-HEPES. The pH was adjusted to 7.4 with 1 N NaOH. A −7 mV correction of the liquid junction potential between the patch pipette and the bath solutions was performed. The recordings were made 10 min after obtaining the whole cell configuration in order to allow the current to stabilize and achieve adequate diffusion of

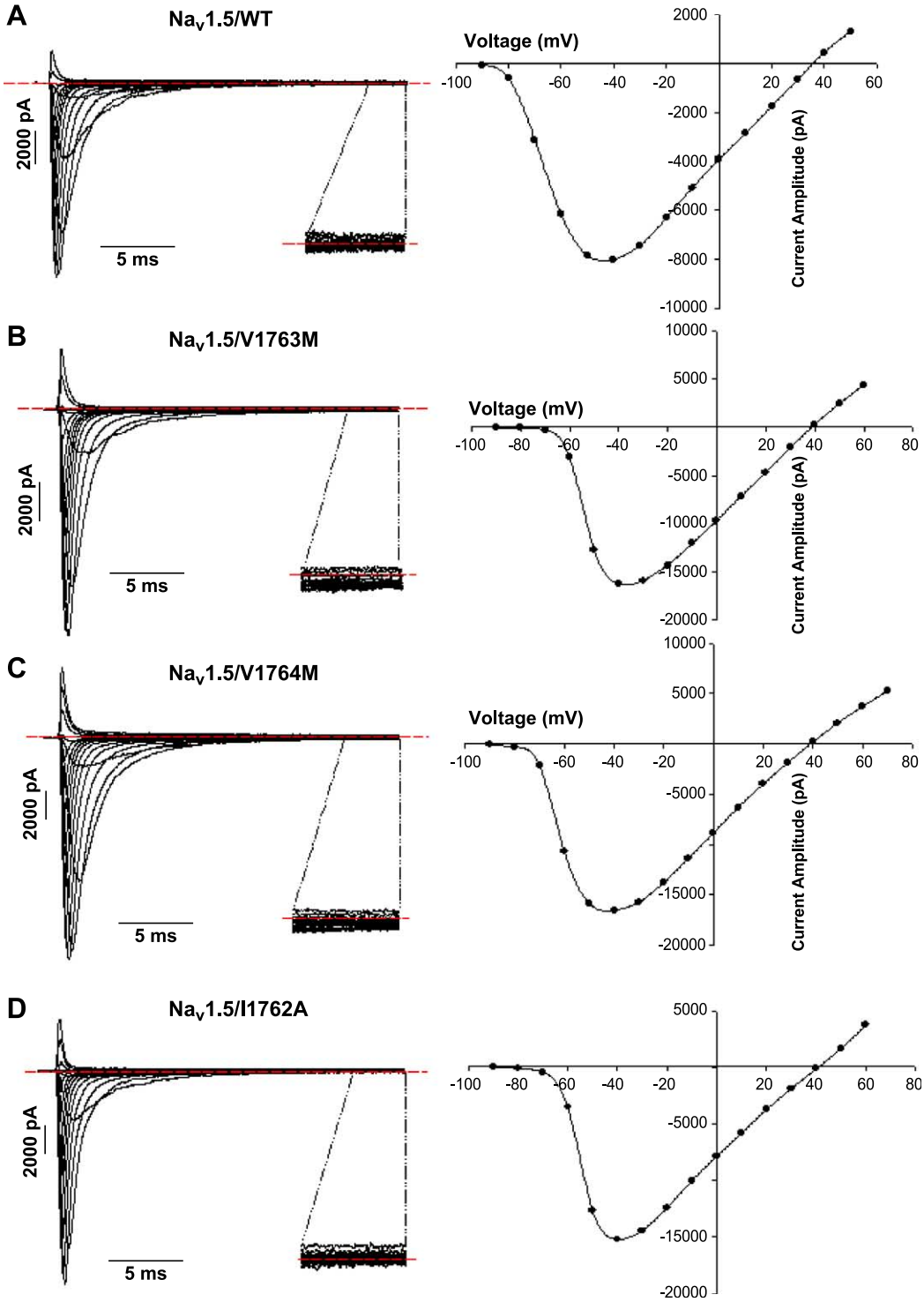

Fig. 4. The left panels represent a family of whole-cell sodium current traces from $Na_v1.5$/WT (A), $Na_v1.5$/V1763M (B), $Na_v1.5$/V1764M (C) and $Na_v1.5$/VI1762A (D) expressed in the tsA201 cell line. Currents were generated from a holding potential of −140 mV from −80 to +50 mV for 30 ms in 10 mV increments. The dashed line represents the zero current. The presence of the residual current is highlighted in the insets. The right panels represent the current–voltage (I/V) relationships of the corresponding current traces shown on the left panels.

the contents of the patch electrode. All the recordings were made in the following order: *I/V* curve, steady-state inactivation and recovery from inactivation. Experiments were carried out at room temperature (22–23 °C). Tetrodotoxin and lidocaine were purchased from Sigma (St. Louis, MO, USA).

2.7. Statistical analysis

Data are expressed as mean±standard error of the mean (S.E.M.). When indicated, a *t*-test was performed using statistical software in SigmaPlot (Jandel Scientific Software, San Rafael, CA, USA). Differences were deemed significant at a p value <0.05.

3. Results

3.1. Patient characteristics

The newborn was referred to our institution due to prenatal bradycardia and postnatal 2:1 atrioventricular (AV) block (Fig. 1A) and torsade de pointes ventricular tachycardia (Fig. 1B). The patient was noted to have intermittent bradycardia (heart rate 74 beats per minute) in late gestation and was delivered at the 38th week of gestation with an uneventful course. At the age of 3 days, bradycardia due to an extremely prolonged QT interval and a 2:1 AV block was noted. At the age of 6 and 8 days, the newborn experienced torsade de pointes ventricular tachycardia that could be converted by intravenous lidocaine or DC conversion. He was referred to our institution at the age of 8 days. The electrocardiogram showed a prolonged QT interval (corrected QT interval=611 ms, heart rate 72/min) and 2:1 AV conduction. The diagnosis of congenital LQTS with AV block and ventricular tachycardia was made and we started intravenous lidocaine infusion after titrating the dose to 40 μg/kg/min. The corrected QT interval was shortened (corrected QT interval=588 ms, heart rate 108/min) and 1:1 AV conduction resumed. Isoproterenol shortened the QT interval further (corrected QT interval=515 ms, heart rate 123/min). Audio and visual evoked potential tests and screening for anti-Ro, anti-La and anti-nuclear antibodies were all negative. The baby received mexiletine only, and when the dosage was increased to 42 mg/kg/day the QT interval was shortened with 1:1 AV conduction (corrected QT interval=518 ms) (Fig. 1C). He rarely experienced AV block without ventricular tachycardia after discharge. At the age of 5 months, he was found to have 2:1 AV block again (corrected QT interval=589 ms, heart rate 62/min) but after adjusting the mexiletine to 32.5 mg/kg/day, the 1:1 AV conduction resumed (corrected QT interval=496 ms, heart rate 114/min). No evidence of gastrointestinal disturbances, irritability or hepatic dysfunction was noted after the initiation of high mexiletine therapy. The family study showed an extremely prolonged QT interval only in the proband and borderline QT interval prolongation in the mother and elder sister (Fig. 2). At age 10 months, ventricular tachycardia recurred after omitting three doses of drugs. He was successfully resuscitated and propranolol (2 mg/kg/day in three doses) was added. However, he died suddenly at home at age 14 months during an episode of acute upper respiratory tract infection.

3.2. SCN5A mutation

SSCP analyses of KVLQT1 and HERG revealed no abnormal conformers. An aberrant band was found in exon 28 of SCN5A only in the proband (Fig. 3A). This

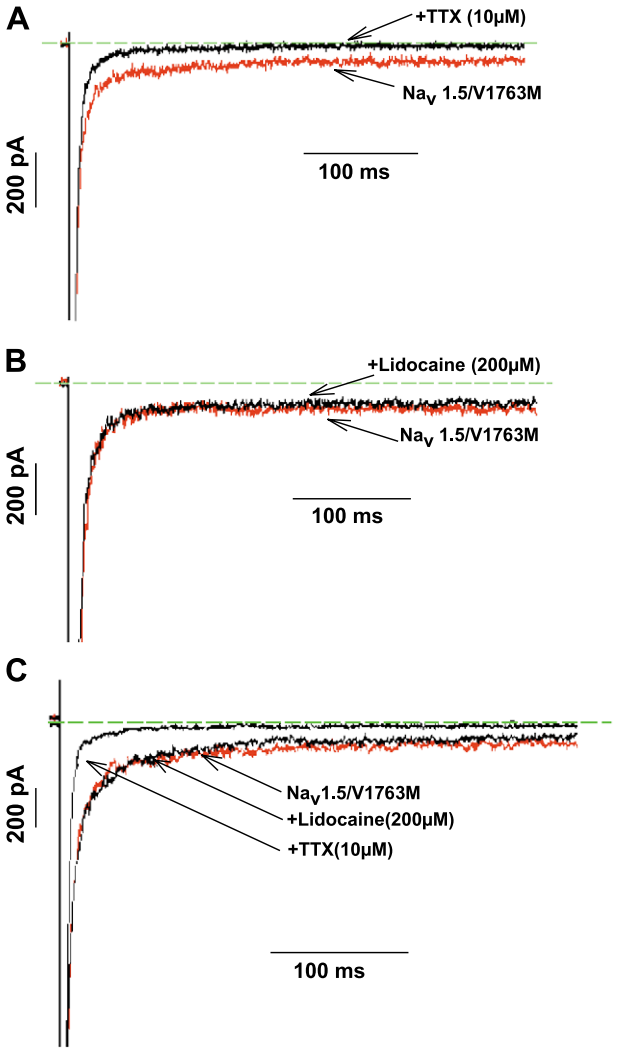

Fig. 5. Panel (A) shows the effect of 10 μM tetrodotoxin on the sodium current recorded from cells expressing $Na_v1.5$/V1763M. Panel (B) shows the effect of 200 μM lidocaine on the sodium current recorded from cells expressing $Na_v1.5$/V1763M. The sodium currents were recorded from a HP =−140 mV to a voltage test of −30 mV before and after adding 10 μM tetrodotoxin. Panel (C) shows the effect of adding 10 μM tetrodotoxin in the presence of 200 μM lidocaine. Note that the residual current was reduced only in the presence of tetrodotoxin. In all panels, the dashed lines represent the zero current.

abnormality was absent in the other 10 family members, including the parents. Bidirectional sequencing of the subsequent aberrant DNA fragments revealed a single base transition (G→A) at position 5287, which was expected to cause a nonconservative change from a valine (GTG) to a methionine (ATG) at codon 1763 in IVS6 of

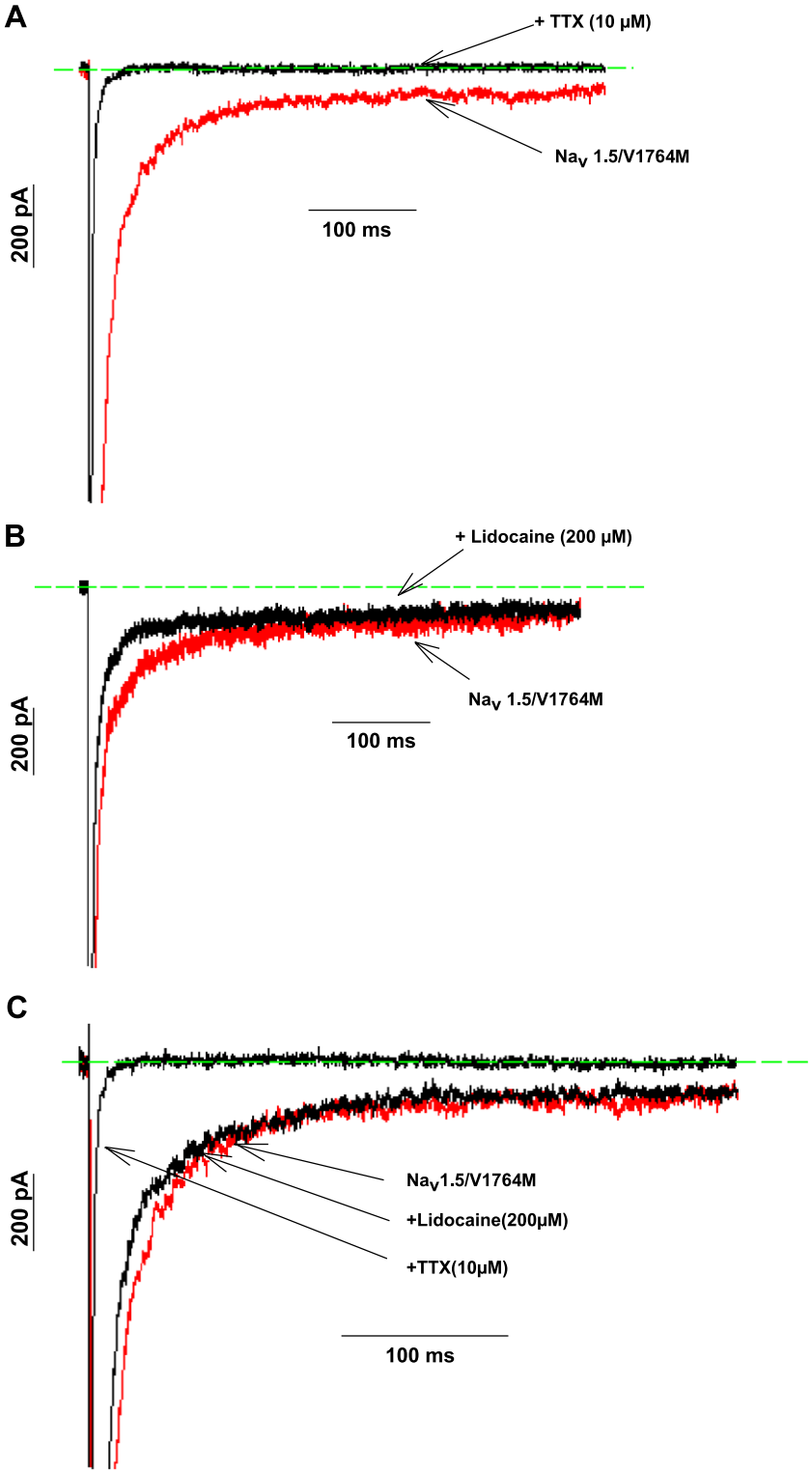

Fig. 6. Panel (A) shows the effect of 10 μM tetrodotoxin on the sodium current recorded from cells expressing $Na_v1.5$/V1764M. Panel (B) shows the effect of 200 μM lidocaine on the sodium current recorded from cells expressing $Na_v1.5$/V1764M. Sodium currents were recorded from a HP =−140 mV to a voltage test of −30 mV before and after adding 10 μM tetrodotoxin. Panel (C) shows the effect of adding 10 μM tetrodotoxin in the presence of 200 μM lidocaine. Note that the residual current was reduced in the presence of tetrodotoxin. In all panels the dashed lines represents the zero current.

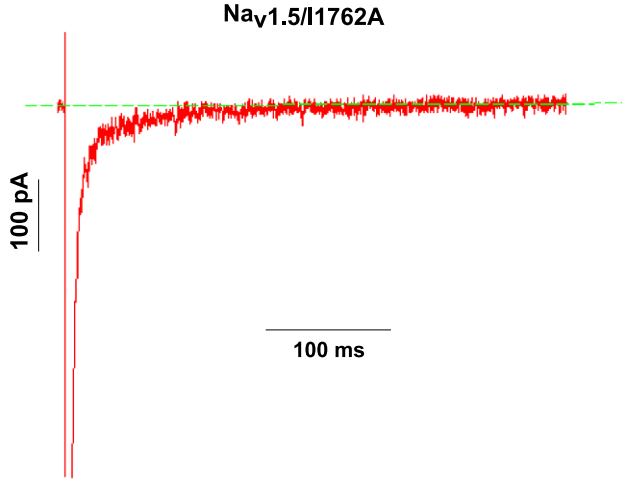

Fig. 7. The sodium current from Na$_v$1.5/I1762A was recorded from a HP =−140 mV to a voltage test of −30 mV. The dashed line represents the zero current. Note the absence of residual current in this mutant sodium channel.

Na$_v$1.5. The patient was heterozygous for this substitution (Fig. 3B). We also found four silent DNA changes that would not alter the amino acid sequence: (1) A to C at position 357, (2) T to C at nucleotide position 840, (3) A to G at position 3080 and (4) C to T at position 5457. The first three of these changes may be benign polymorphisms unique to Taiwan population as they were seen in both the LQTS patient and the normal control population (frequency 100%). However, C5457T was a rare genetic variant as it was seen only in the LQTS patient and was not detected in our normal control population (frequency 0.4%). The frequency of this single nucleotide polymorphism (SNP) is 0.46 in Japan and 0.12 in the North America [11,12].

3.3. Electrophysiological properties of the mutation

Macroscopic sodium currents were recorded from tsA201 cells expressing wild-type (Na$_v$1.5/WT) and mutant channels (Na$_v$1.5/V1763M, and neighboring Na$_v$1.5/V1764M and Na$_v$1.5/I1762A) co-transfected with the β_1 subunit (see Methods for more details on identifying cells expressing the β_1 subunit) (Fig. 4). Sodium channel densities were not significantly different between the wild type and mutants (WT: 608.6±30 pA/pF, n=10; V1764M: 788.4±20 pA/pF, n=14; V1763M: 728±58 pA/pF, n=9 and I1762A: 661.5±40 pA/pF, n=8), suggesting that all mutant channels expressed equally. The resulting sodium currents showed fast activation and inactivation kinetics. However, the mutant Na$_v$1.5/V1763M and Na$_v$1.5/V1764M channels were characterized by the presence of a persistent inward sodium current of about 1% of the maximum current measured at −30 mV (1.1±0.1, n=11, and 1.3±0.1, n=13, respectively). Mutant Na$_v$1.5/I1762A did not exhibit any residual currents. The current–voltage (I/V) relationship of all the mutant channels was shifted to more positive potentials.

The persistent sodium current recorded from the Na$_v$1.5/V1763M and Na$_v$1.5/V1764M mutant channels dropped to almost zero in the presence of 10 μM tetrodotoxin, a specific sodium channel blocker (Fig. 5A for Na$_v$1.5/V1763M and Fig. 6A for Na$_v$1.5/V1764M). Lidocaine at 200 μM was ineffective in reducing the persistent sodium current (Figs. 5B and 6B). Tetrodotoxin (10 μM) in the presence of lidocaine totally inhibited the residual current (Figs. 5C and 6C). No residual current was recorded with mutant Na$_v$1.5/I1762A (Fig. 7).

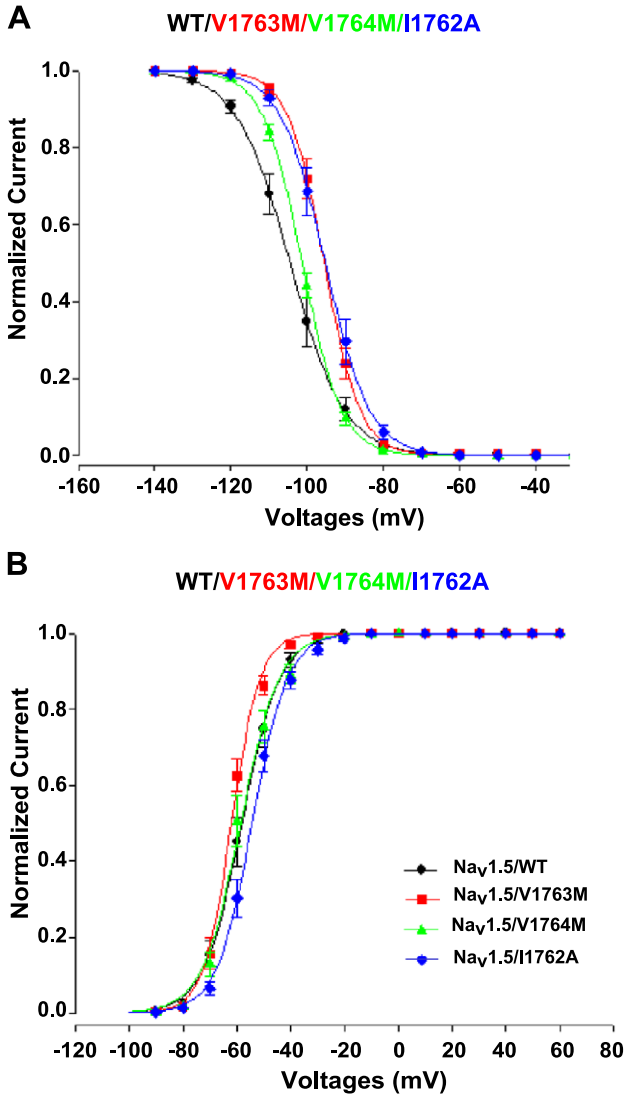

Fig. 8. Voltage dependence of the steady state activation (Gv) (A) and steady state inactivation (h_∞) (B) of Na$_v$1.5/WT, Na$_v$1.5/V1763M, Na$_v$1.5/V1764M, Na$_v$1.5/I1762A. Data points of steady state inactivation were fitted using a Boltzmann equation with K_v and $V_{1/2}$ representing respectively 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 a −140 mV holding potential. See Table 1 for parameters.

Table 1
Activation, inactivation and recovery-from-inactivation parameters of wild-type and mutant sodium channels

	Activation		Inactivation		Recovery from inactivation
	$V_{1/2}$ (mV)	K_v	$V_{1/2}$ (mV)	K_v	τ_{rec} (ms)
WT (n=9)	−58.4±1.6	−6.5±0.3	−107.0±0.9	5.2±0.2	6.1±0.7
V1763M (n=6)	−62.2±1.2	−4.7±0.5*	−95.5±1.0*	4.6±0.1	4.1±0.4*
V1764M (n=7)	−59.1±1.7	−6.6±0.5	−101.0±0.7*	5.1±0.2	7.7±0.6
I1762A (n=7)	−54.3±1.9	−6.0±0.6	−95.2±2.3*	4.7±0.1	3.3±0.3*

n: indicates the number of cells tested.
*: indicates statistically significant differences.

Steady-state activation and inactivation curves were also studied (Fig. 8 and Table 1). A shift of the steady state inactivation curve of about 8 mV (p<0.05) toward more positive voltages was recorded for the V1763M mutant ($V_{1/2v}^{Na1.5/V1763M}$=−95.5±1 mV, n=6 versus $V_{1/2v}^{Na1.5/WT}$=−107.47±1 mV, n=9); the slope factor was not significantly affected. The Gv or steady-state activation curves of all the mutant sodium channels showed no significant shifts (Table 1). However, the slope factor of the steady state activation curve of mutant V1763M was significantly steeper ($kv_v^{Na1.5/V17763M}$=4.7±0.5 mV, n=6 versus $kv_v^{Na1.5/WT}$=6.5±0.3 mV, n=9, p<0.05). The recovery from inactivation of V1763M and I1762A was also significantly faster than that of wild type (Table 1).

4. Discussion

The age of onset in congenital LQTS varies, but patients with onset as early as the prenatal stage are rare. This study identified a novel heterozygous SCN5A mutation at IVS6 of $Na_v1.5$ that resulted in LQTS with ventricular arrhythmia and AV conduction block during the perinatal stage. The clinical improvement after intravenous lidocaine or oral mexiletine strongly suggested Na channelopathy. The expression study confirmed the functional abnormality of the mutant Na channel.

It is generally agreed that patients with LQTS and prenatal or neonatal onset have a poor prognosis [4–6]. From the literatures, 27 patients of LQTS were found to have prenatal onset of arrhythmias, tachycardia in 3 and bradycardia in all. In 14 patients with mutation analysis, the mutations were identified in HERG (n=6), KCNQ1 (n=5), KVLQT1 (n=1), SCN5A (n=1) and mixed sites (n=1) [4–6,13–15]. Once the mutation responsible for LQTS has been determined, the therapy could be tailored to the specific ion channel defect. For example, Na channel blockers, mexiletine and lidocaine, may prevent the repetitive opening of the channel, shorten the QT interval and normalize the morphology of the T wave in the animal LQT3 models [16]. In patients with LQT3, mexiletine can shorten the corrected QT intervals and the QT interval in response to increases in heart rate [17]. Mexiletine has also been shown to be effective in reducing dispersion and preventing torsade de pointes in animal models of LQT2 and LQT3 [16]. In clinical studies of LQT3, the patients ranged from 3 to 44 years of age and the mexiletine dosage ranged from 12 to 16 mg/kg/day [18]. Our patient may be the youngest so far reported with LQTS in whom the atrioventricular block and ventricular tachycardia were initially successfully treated by lidocaine and mexiletine. The effective dosage range for mexiletine (30 to 40 mg/kg/day) was much higher than the suggested range for children (7.5 to 15 mg/kg/day) [19]. A dosage of 15 to 25 mg/kg/day has been reported for two pediatric patients with refractory supraventricular tachycardia [20]. No adverse effects due to high doses of mexiletine were noted. We also observed in vitro persistent tetrodotoxin sensitive currents from the mutant of this patient. The region of this mutation, domain IVS6 of $Na_v1.5$, contains the binding site of antiarrhythmic drugs [21]. In the expressed tsA201 cells, lidocaine at 200 μM failed to effectively reduce the persistent sodium current a dose that is expected to block the persistent tetrodotoxin sensitive currents recorded from other LQT3 mutations [22,23]. This discrepancy between the in vivo and in vitro effects of lidocaine remains unclear. We suspect that the in vitro concentration to effectively suppress the mutant Na channel in over expressed tsA201 cells may be much higher than in vivo. But, further studies are mandatory to elucidate the mechanisms.

Since isoproterenol further shortened the QT interval and previous studies indicated potential adverse effects of β-blockers in LQT3, a β-blocker was not initially given. Shimizu et al. [24] found that β-adrenergic stimulation by isoproterenol might induce torsade de pointes by increasing transmural dispersion of repolariza-

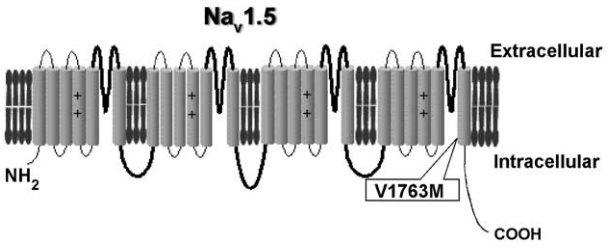

Fig. 9. Diagrammatic representation of the human cardiac sodium channel displaying the location of the mutation identified in this study.

tion in LQT1 and LQT2 models, whereas it suppressed torsade de pointes by decreasing the dispersion in a LQT3 model [24]. They therefore suggested that β-blockers are protective in LQT1 and LQT2 but may facilitate torsade de pointes in LQT3 models of long QT syndrome. Propranolol was added to the treatment regimen of our patient at age 10 months. The patient died suddenly at home 4 months later. We do not have adequate information to determine the cause of the sudden death of the patient.

Our electrophysiological data confirmed that the identified SCN5A mutant (V1763M) was associated with a persistent Na current, resulting in LQTS. This is consistent with previous reports indicating that most mutations in SCN5A causing LQTS had an enhanced persistent Na current [25–29]. In addition, we also demonstrated a persistent Na current for the neighboring mutation (V1764M). The persistent currents recorded from the V1763 and V1764M mutants could be suppressed by tetrodotoxin. However, the other neighboring I1762A mutant was not associated with a persistent Na current. But, a positive shift of the steady-state inactivation was found in all. Along with either a steeper activation curve (V1763M) or a faster recovery from inactivation (V1763M and I1762A), the voltage-range over which "window current" will be increased. The balance of repolarizing and depolarizing currents may be altered and lead to prolonged repolarization. An M1766L mutation identified from an infant with congenital LQTS that was in close proximity to 1763 and 1764 positions similarly has a persistent Na current due to a positive shift of the steady state inactivation [29]. That patient initially responded to propranolol and mexilentine, but died suddenly at the age of 16 months. Another I1768V mutant at IVS6 of $Na_v1.5$ identified from an adult with congenital LQTS was associated with only faster recovery from inactivation, but not a persistent Na current or inactivation shift [30]. The IVS6 of $Na_v1.5$ is critical for the inactivation kinetics of Na currents [31,32]. The positions 1762, 1763, 1764 (our study), 1766 [29] and 1768 [30] are all amino acid residues in IVS6 and close to the junction between IVS6 and C-terminus of $Na_v1.5$ (Fig. 9). The mutations at these points tend to have a positive shift of inactivation, steeper activation or faster recovery from inactivation. A significant persistent residual current was recorded with mutations at positions 1763, 1764 and 1766. The known C-terminus $Na_v1.5$ mutants on the other hand are associated with a negative shift of steady-state inactivation and intermediate inactivation [2].

In conclusion, our study suggests that the V1763M-SCN5A channel dysfunction may contribute to a persistent inward current due to altered inactivation and activation kinetics of Na current in cardiac myocytes and clinically congenital LQTS with perinatal onset of the arrhythmias and a poor prognosis. The expression of the mutations, including I1762A, V1763M and V1764M, showed a similar spectrum of electrophysiological properties as other reported mutants of IVS6 of $Na_v1.5$ (M1766L and I1768V) in controlling the inactivation kinetics of Na channels.

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