Homozygous SCN5A Mutation in Long-QT Syndrome With Functional Two-to-One Atrioventricular Block

J.M. Lupoglazoff, T. Cheav, G. Baroudi, M. Berthet, I. Denjoy, B. Cauchemez, F. Extramiana, M. Chahine, P. Guicheney

Abstract—Heterozygous mutations in genes encoding cardiac ionic channel subunits KCNQ1, HERG, SCN5A, KCNE1, and KCNE2 are causally involved in the dominant form of long-QT syndrome (LQTS) while homozygous mutations in KCNQ1 and KCNE1 cause LQTS with or without congenital deafness. In addition, two homozygous HERG mutations have been associated with severe LQTS with functional atrioventricular conduction anomalies in young children. A 2:1 atrioventricular block (AVB) with a major QTc prolongation (526 ms) was evidenced in a 5-year-old boy referred for syncope and seizure. LQTS was diagnosed and beta-blocking therapy initiated leading to normal atrioventricular conduction. Electrophysiological study provided support that location of the AVB was infra-Hisian. DNA analysis was performed in the proband and in asymptomatic family members. A novel missense mutation, V1777M, in the early C-terminal domain of SCN5A was identified. The proband was homozygous while the parents and two siblings were heterozygous carriers. Homozygote and heterozygote expression of the mutant channels in tsA201 mammalian cells resulted in a persistent inward sodium current of 3.96±0.83% and 1.49±0.47% at 30 mV, respectively, which was dramatically reduced in the presence of tetrodotoxin. This study provides the first evidence for a homozygous missense mutation in SCN5A and suggests that LQTS with functional 2:1 AVB in young children, a severe phenotype associated with bad prognosis, may be caused by homozygous or heterozygous compound mutations not only in HERG but also in SCN5A. The full text of this article is available at http://www.circresaha.org. (Circ Res. 2001;89:e16-e21.)

Key Words: arrhythmias ■ clinical genetics ■ ion channels

The congenital long-QT syndrome (LQTS) is a potentially lethal cardiac disease caused by mutations in specific ion channels. Heterozygosity for a mutation in the potassium channel genes HERG (LQT2) and KCNE2 (LQT6) causes defects in the rapid component of the delayed rectifier Ikr, whereas mutations in KCNQ1 (LQT1) and KCNE1 (LQT5) cause defects in the slow component of the delayed rectifier Iks.1 Mutations in SCN5A cause a persistent cardiac sodium current that is responsible for LQT3.2 This form has been associated with a lower rate of cardiac events but a higher rate of lethal events.3 Homozygosity for mutations in KCNQ1 and KCNE1 have been associated with the recessively inherited Jervell and Lange-Nielsen syndrome, in which QT prolongation is associated with a bilateral neurosensitive deafness.4–6 Nevertheless, several LQT1 recessive variants without deafness have also been described.7,8 Recently, homozygosity for HERG mutations associated with atrioventricular (AV) conduction disturbances have been reported.9,10 We describe the first case of an LQTS patient with functional 2:1 AV block (AVB) due to a homozygous missense mutation in SCN5A.

Materials and Methods

Clinical Data

The proband of a 9-member family was referred for syncope. The father originated from Senegal and the mother from Guinea without any known consanguinity. Both parents and the 7 children underwent clinical evaluation and cardiovascular examination, including a 12-lead ECG (ECG) and a 24-hour Holter recording. QT interval was measured on the surface ECG in lead II and corrected for heart rate (QTc) using Bazett’s formula. Blood samples were obtained after written consent in accordance with the protocol approved by the local ethics committee following the guidelines set down by the Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale de la Pitié-Salpêtrière (Paris, France). An electrophysiological study was performed in the proband during follow-up under nadolol (50 mg/m² per day) and sedation to further document the mechanism and location of the AVB. One quadriolar and one octapolar 6F electrode catheters were introduced via the right femoral vein and positioned respectively in the high right atrium and in the His bundle area. Endocavitary signals were acquired through a Prucka system and filtered (30 to 500 Hz). Stimulation was performed at twice the diastolic threshold level with decremental pacing using the ramp technique with a progressive decrement of the cycle length by 10-ms steps.
Mutagenesis and Vector Construction

The mutant hH1/V1777M was generated according to QuickChange site-directed mutagenesis kit instruction manual from Stratagene. The mutation was performed using the following mutagenic sense and antisense primers:

\[ 5'\text{-CTGGAGAATTCATGAGGACGATGCTGAAGTTCCAG-3'} \]
\[ 5'\text{-GTGCTCTCCTCCCGTGGCCACGGAGGAGAGCAC-3'} \]

(for hH1/V1777M)

The underlined nucleotides represent the mutation sites. The mutant and wild-type hH1 were cloned in a pcDNA1 vector and purified using Qiagen columns. The human sodium channel \( \beta_1 \) subunit and CD8 were constructed in a bisistronic CD8-pIRES-\( \beta_1 \) vector. By the use of anti-CD8-a-coated beads (Dynabeads M-450 CDS-a), cells expressing the \( \beta_1 \) subunit were visualized by light microscopy according to the manufacturer’s instructions (Dynal).

Transfections of tsA201 Cell Line

The mammalian cell line tsA201 was derived from human embryonic kidney HEK293 cells by stable transfection with SV40 large T antigen. Cells were grown in high-glucose DMEM (Dulbecco’s modified Eagle’s medium) supplemented with 10% FBS (fetal bovine serum), L-glutamine (2 mmol/L), penicillin G (100 U/mL), and streptomycin (10 mg/mL) (Gibco BRL Life Technologies). Cells were incubated in a 5% CO2-humidified atmosphere. The tsA201 cells were transfected in a bisistronic CD8-pIRES-\( \beta_1 \) vector, with the use of anti-CD8-a-coated beads (Dynabeads M-450 CDS-a), cells expressing the \( \beta_1 \) subunit were visualized by light microscopy according to the manufacturer’s instructions (Dynal).

Patch-Clamp Method

Macroscopic sodium currents from transfected cells were recorded using the whole-cell configuration of the patch-clamp technique.12,13 Patch electrodes were made from 8161 Corning glass and coated with Sylgard (Dow-Corning) to minimize their capacitance. Low-resistance electrodes (<0.8 MΩ) were used, and a routine series resistance compensation of an Axopatch 200 amplifier was performed to values >80% to minimize voltage-clamp errors. Voltage-clamp command pulses were generated by microcomputer using pCLAMP software version 5.5 (Axon Instruments). Sodium currents were filtered at 5 kHz. Experiments were performed 10 minutes after obtaining the whole-cell configuration to allow the current to stabilize and reduce the experimentally caused shift of gating.14

Solutions and Reagents

For whole-cell recording, the patch pipette contained (in mmol/L) NaCl 35, CsF 105, EGTA 10, and Cs-HEPES 10 (pH 7.4). The extracellular solution contained (in mmol/L) NaCl 150, KCl 2, CaCl2 1.5, MgCl2 1, glucose 10, and Na-HEPES 10 (pH 7.4). A correction of the liquid junction potential of \(-7 \text{ mV} \) between patch pipette and

Solution of the Liquid Junction Potential of 2 Cells Were Transfected Using the Calcium Phosphate Method, with 10

Polymerase chain reaction–single-strand conformation polymorphism (PCR-SSCP) analysis was performed as previously described at 7°C and 25°C.12 When abnormal patterns were observed, PCR products were reamplified and sequenced by the dideoxynucleotide chain termination method with fluorescent dideoxynucleotides on an ABI-Prism 377 DNA sequencer (Applied Biosystems).

Figure 1. Pedigree of family 16405. The homozygous carrier of the SCN5A mutation is shown by a solid symbol, heterozygous carriers by half-filled symbols, females by circles, and males by squares. Age, QT, and heart rate values recorded in lead II of the surface ECG at 25 mm/s are given. ECGs of the proband showed a 2:1 AV block with a major QTc prolongation (a) and disappearance of AV conduction abnormalities after beta-blockade (b). SD indicates sudden death.

Statistical Analysis

Data are expressed as mean±SEM. When indicated, \( t \) test analysis was assessed using statistical software in SigmaPlot (Jandel Scientific Software). Differences were considered to be significant at a value of \( P<0.05 \).

Results

Phenotypic Characteristics of the Family

The index patient was referred at 5 years of age for syncope and seizure, which occurred while playing at home in the absence of any drug intake. The initial ECG, recorded a few hours after admission, showed a ventricular bradycardia at 50 bpm with a 2:1 AVB, a major QT prolongation (QT=575 ms; RR=1200 ms; QTc=526 ms), and normal PR (120 ms) and QRS (82 ms) intervals during 1:1 AV conduction (Figure 1, II-5, ECGa). The Holter recording confirmed the QT prolongation and showed frequency-dependent conduction anomalies with episodes of 2:1 AVB when atrial rate increased above 100 bpm preceded by intermittent bundle-branch block. The first atrial impulse was blocked after the end of the T wave, and during the subsequent 2:1 AVB, the P wave fell on the descending phase of the T wave. During periods of 1:1 AV conduction, T-wave morphology was abnormal with a delayed T-wave onset of high amplitude without any notches. Treatment with the beta-blocking agent nadolol was instituted (50 mg/m² per day). The atrial rate decreased to 80 bpm leading to the disappearance of the functional AVB (Figure 1, II-5, ECGb). Audiogram showed normal bilateral hearing.

Episodes of 2:1 AVB were found on a Holter recording performed after 12 months of nadolol (50 mg/m² per day) at a triggering atrial rate of 100 bpm. To further document the mechanism of the AVB, the patient underwent an electrophysiological study. Under basic conditions, the PR, RR, QRS, and QT values were 160, 695, 80, and 390 ms, respectively. The AH and HV intervals were 90 and 38 ms, respectively. Atrial pacing constantly induced an infra-Hisian
2:1 AVB for a pacing cycle length of 540 to 550 ms (Figure 2). During 2:1 AVB, the AH interval remained constant at 110 ms, and the HV interval for conducted QRS complexes was 40 ms. In all cases, His bundle activity was detected well after the end of the T wave (90 ms) on the surface ECG.

Bundle-branch block repeatedly occurred before episodes of 2:1 AVB, corresponding to either a left anterior hemiblock or a left anterior hemiblock associated with a right bundle-branch block. The right ventricular effective refractory period at the apex was 280 ms at a basic cycle length of 650 ms, and 240 and 230 ms for stimulation cycle lengths of 500 and 400 ms, respectively. This discrepancy between relatively short right ventricular refractory periods and the value of the cycle length promoting 2:1 AVB, together with the occurrence of bundle branch conduction defect, was in favor of a location of the block in the Purkinje system rather than at a more distal level. No ventricular arrhythmia was induced despite delivery of 3 extrastimuli at the apex and the right ventricular infundibulum during sinus rhythm and ventricular pacing at cycle lengths of 400 and 500 ms, respectively. A unipolar ventricular endocardial pacemaker was implanted at the end of the electrophysiological procedure for ventricular overdrive pacing at 100 bpm.

One sibling experienced intrauterine death at a gestational age of 28 weeks, but no autopsy was performed. None of the other family members experienced any symptoms related to LQTS. The father (I-1), the mother (I-2), and one sibling had borderline QTc intervals with normal heart rates (Figure 1). The QRS and PR intervals and the heart rate of the parents and the siblings were within normal range with no conduction anomalies evidenced at Holter recording.15

Identification of the SCN5A Mutation
SSCP analysis of KCNQ1, HERG, and KCN1 did not reveal any abnormal conformer. In contrast, aberrant bands were found in exon 28 of SCN5A in the affected subject (II-5) as well as in the parents (I-1 and I-2), and 2 of the siblings (II-1 and II-2) (Figure 3A). The SSCP abnormalities were absent in 150 Caucasian unrelated individuals. Bidirectional sequencing of the subsequent aberrant DNA fragments revealed a single base transition (G→A) at position 5329, which is expected to cause a nonconservative change from a valine (GTG) to a methionine (ATG) at codon 1777 within the C-terminal domain of the sodium channel (Figures 3C, 3D, and 4). The parents and siblings II-1 and II-2 were heterozygous for this substitution (sequence not shown), whereas the proband was homozygous. In addition, SSCP analyses revealed a polymorphism in exon 12. The sequence variation, 1673A→G, was a frequent polymorphism resulting in an amino-acid substitution, His558Arg. The proband was homozygous for this polymorphism as well as for the three...
LQT3 mutations in the early C-terminus of the cardiac sodium channel protein. Topological model of the channel (A) is illustrated with location of 4 mutations associated with LQT3. V1777M reported here is boxed; the other 3, E1784K, S11787N, and D1790G, have been previously reported. Amino-acid sequence alignments of SCN5A with related sodium channel sequences (B) are shown (SCN5A, human heart [M77235]; SCN4A, human skeletal muscle [M81758]; SCN1A, human neuronal type I [AF225985]; SCN2A, human neuronal type II [M94055]; SCN3A, human neuronal type III [AF229887]; SCN8A, human type VIII [XM006838]; Eel, eel electrophorax [M22282]). Gen-Bank accession numbers are given in square brackets. First line, amino-acid substitutions corresponding to the 4 above-mentioned LQT3 mutations.

Figure 4. SCN5A mutations in the early C-terminus of the cardiac sodium channel protein. Topological model of the channel (A) is illustrated with location of 4 mutations associated with LQT3. V1777M reported here is boxed; the other 3, E1784K, S11787N, and D1790G, have been previously reported. Amino-acid sequence alignments of SCN5A with related sodium channel sequences (B) are shown (SCN5A, human heart [M77235]; SCN4A, human skeletal muscle [M81758]; SCN1A, human neuronal type I [AF225985]; SCN2A, human neuronal type II [M94055]; SCN3A, human neuronal type III [AF229887]; SCN8A, human type VIII [XM006838]; Eel, eel electrophorax [M22282]). Gen-Bank accession numbers are given in square brackets. First line, amino-acid substitutions corresponding to the 4 above-mentioned LQT3 mutations.

Functional Properties of the V1777M Mutation In Vitro
Macroscopic sodium currents were recorded from tsA201 cells expressing wild-type (hH1/WT) and mutant channels (hH1/V1777M), both cotransfected with the β1 subunit (Figures 5A and 5B). The resulted sodium channels were functional with fast activation and inactivation kinetics. However, sodium currents from homozygote (hH1/V1777M) and heterozygote (hH1/V1777M+hH1/WT) expression systems were characterized by the presence of a significant persistent inward sodium current of 3.96 ± 0.66 nA/pA at 120 mV, before and after adding TTX. Steady-state voltage dependence activation and inactivation curves in normal and mutated sodium channels fitted to a Boltzmann distribution: hH1/WT + β1 (#, dotted lines), hH1/V1777M + hH1/WT + β1 (+, straight lines), and hH1/V1777M + β1 (•, dashed lines) (D).

Discussion
Heterozygous mutations or deletions of SCN5A, the gene that encodes the cardiac sodium channel, are causally involved in LQT3, Brugada syndrome, and isolated cardiac conduction defects.16 In the present study, we describe a novel LQT3 missense mutation, V1777M, in the cytoplasmic C-terminal part of the protein, identified at the homozygous state in a symptomatic child with AV conduction disturbances.

Functional analysis show that most LQT3 mutants fail to inactivate completely and promote sustained inward sodium current resulting in ventricular repolarization prolongation.17–20 The affected residue (Val 1777) is located within a highly conserved acidic domain located in the early carboxy terminal fragment of the sodium channel protein where three other LQT3 mutations, E1784K,12,21 D1790G,13,22 and S1787N,1 have been reported. They all affect very conserved residues among the sodium channel gene family (Figure 4).

Two carboxy terminal mutants have been expressed, D1790G13,22 and E1784K12,21 The D1790G mutation has first been shown to affect the voltage dependence of sodium current inactivation by disrupting the α-β interaction without promoting sustained inward sodium current.22 However, a persistent inward sodium current was found reported for most of the LQT3 mutations.16 This persistent current was associated for the 3 C-terminal mutations expressed in the presence of β1 subunit with a shift of inactivation toward more negative potentials.2,13,21,22 Reports in the literature on SCN5A mutation expression refer to models using cells expressing either wild-type channels or mutated channels separately, thus mimicking in fact the effects of homozygous mutations. In heterozygous carriers, both the wild-type and the mutated channels coexist in the
Voltage Dependence of Steady-State Activation and Inactivation Parameters of Sodium Currents From Wild-Type, Homozygote, and Heterozygote V1777M

<table>
<thead>
<tr>
<th>SCN5A Channel Type</th>
<th>Inactivation Curve</th>
<th>Activation Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$V_{1/2}, \text{mV}$</td>
<td>$K_v, \text{mV}$</td>
</tr>
<tr>
<td>hh1/WT</td>
<td>-92.47±1.1 (n=11)</td>
<td>5.4±0.3 (n=11)</td>
</tr>
<tr>
<td>hh1/V1777M</td>
<td>-104.9±1.5* (n=5)</td>
<td>5.3±0.0.7† (n=5)</td>
</tr>
<tr>
<td>hh1/WT + hh1/V1777M</td>
<td>-98.12±3.4* (n=10)</td>
<td>5.3±0.16† (n=10)</td>
</tr>
</tbody>
</table>

For steady-state inactivation, sodium currents were triggered by 500-ms prepulse ranging from -140 to -20 mV.

*P<0.05 compared with wild type (WT).
†Not significant.

same patient. Our coexpression experiments were performed to better characterize the effect of the V1777M mutation according to the different genetic status of the family. We observed that the residual sodium current was more important in the homozygote model compared with the heterozygote one, correlating with the differences in phenotype in these family members. Indeed, the affected phenotype characterized by a major QTc prolongation and the 2:1 AVB was found only in the proband, possibly related to the electrophysiological effect of the mutation at the homozygote state. In contrast, the electrophysiological consequence of mutated heterozygote channels produced a mild QT prolongation and no 2:1 AVB. Carriers of the heterozygous V1777M mutation display a mild form of LQTS, but they had longer QTc intervals (415 to 442 ms) compared with noncarriers (396 to 408 ms). Both parents and 2 siblings were asymptomatic with borderline QTc and no history of familial sudden death, suggesting a low penetrance of this mutation at the heterozygote state. Mutation effect on the current varies with experimental conditions such as the expression system or the composition of the extra- and intrasolutions. Nevertheless, it could be noted that in the same experimental conditions, the authors observed a residual current of 6% for D1790G and insD179513 and only 3% for the V1777M mutation. This may explain why the heterozygous carriers of the V1777M mutation have a mild phenotype compared with other LQT3 patients, although no correlation between the effect of the mutation on the sodium current and the duration of the QT interval has been shown so far.

LQTS with 2:1 AVB is rare, with an incidence of 4% reported in pediatric series and a high mortality rate, >50% after 6 months, regardless of the treatment.23 LQTS with AV conduction disturbances has been reported often in infants or young children with a major QTc prolongation, but without any positive familial history.23–28 As in our case, the fast atrial rate, which characterizes pediatric sinus rhythm, can lead to functional AV block in the setting of dramatic prolongation of ventricular repolarization as the P wave falls within the T wave. This form mainly affects neonates and seems to be associated with a worse prognosis.24,28,29 Electrophysiological studies have confirmed the location of such blocks at the ventricular level associated with major prolongation of the refractoriness in the ventricular muscle.25,29 In contrast, our results provide evidence for an infra-Hisian block location in the His-Purkinje system rather than at the ventricular level. This was assessed by the occurrence during decremental atrial pacing of systemized bundle-branch conduction defect before the 2:1 AVB with a His bundle potential occurring in all cases 90 ms after the end of the ventricular repolarization on the surface ECG. This is in accordance with Pruvot et al27 and Gorgels et al,26 who also evidenced at electrophysiological study a block in the His-Purkinje system in patients with 2:1 AVB. Hence, a very long rate-dependent Purkinje/ventricular effective refractory period related to mutations in cardiac ion channel genes and age-related heart rate likely contribute to the development of 2:1 AVB. Our data suggest that the V1777M mutation at the homozygous state can affect both ventricular repolarization and His-Purkinje system, thus producing QT interval prolongation and AV conduction disturbances.

The role of SCN5A in cardiac conduction has been enlightened by Schott et al,30 who identified SCN5A mutations in 2 families with inherited conduction disease. Tan et al31 confirmed this hypothesis by reporting an SCN5A mutation in a family with isolated conduction system disease. Evidence of conduction slowing was found throughout the conduction system with episodes of sinus bradycardia and QRS and PR intervals lengthened but without any QTc interval prolongation. Nevertheless, the potential link between the inherited conduction disease and LQTS remains to be further investigated.

As of today, little is known on the genotype of patients with LQTS and 2:1 AVB. Recent reports of two separate cases of functional 2:1 AVB have been related to homozgyous mutations in HERG.8,10 However, any genetic defect associated with electrophysiological anomalies producing major ventricular repolarization delay could promote such a phenotype. One can speculate that the level of block could also depend on the genotype, with a possible involvement of SCN5A in case of a block in the His-Purkinje system. In addition, in one of the families with homozgyous mutation in HERG, an intrauterine death occurred, as did in our family. Although we did not genotype the fetus, it is conceivable that a homozygous fetus may be at risk of sudden death, in accordance with the occurrence of death very early in life in the series of young children with AVB and LQTS. Considering the discrepancy between the severe ECG phenotype in the probands and the rest of the family members in this study and in two others,8,10 one can speculate that such affected subjects may bear homozygous or heterozygous compound mutations in any one of the LQTS genes with mild functional consequences on the mutated channels in heterozygote carri-
ers. Another explanation could be the coexistence in the proband of an inherited mutation with a low penetrance and a de novo mutation.

Because of their effect on slowing sinus rhythm, beta-blockers have been used to prevent 2:1 AVB, eventually combined with continuous pacing.24 The rationale for using beta-blockers in this study was 2-fold: to slow sinus rate and therefore avoid functional 2:1 AVB and to prevent further adrenergically triggered cardiac events. Indeed, the patient remained asymptomatic during a 12-month follow-up. However, because 2:1 AVB could still be documented on Holter recording despite beta-blocking therapy, a decision was made to implant a pacemaker associated with beta-blocking therapy. Children with prolonged QT interval and persistent functional AVB are at high risk of sudden death and should require permanent pacing and continuous beta-blocking treatment.24,25

In conclusion, this study suggests that LQTs with functional 2:1 AVB in children may be caused by homozygous mutations also in SCN5A and provides the first evidence for a homozygous mutation in SCN5A.

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