De Novo Mutation in the SCN5A Gene Associated With Early Onset of Sudden Infant Death

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Background—Congenital long QT syndrome (LQTS), a cardiac ion channel disease, is an important cause of sudden cardiac death. Prolongation of the QT interval has recently been associated with sudden infant death syndrome, which is the leading cause of death among infants between 1 week and 1 year of age. Available data suggest that early onset of congenital LQTS may contribute to premature sudden cardiac death in otherwise healthy infants.

Methods and Results—In an infant who died suddenly at the age of 9 weeks, we performed mutation screening in all known LQTS genes. In the surface ECG soon after birth, a prolonged QTc interval (600 ms) and polymorphic ventricular tachyarrhythmias were documented. Mutational analysis identified a missense mutation (Ala1330Pro) in the cardiac sodium channel gene SCN5A, which was absent in both parents. Subsequent genetic testing confirmed paternity, thus suggesting a de novo origin. Voltage-clamp recordings of recombinant A1330P mutant channel expressed in HEK-293 cells showed a positive shift in voltage dependence of inactivation, a slowing of the time course of inactivation, and a faster recovery from inactivation.

Conclusions—In this study, we report a de novo mutation in the sodium channel gene SCN5A, which is associated with sudden infant death. The altered functional characteristics of the mutant channel was different from previously reported LQTS3 mutants and caused a delay in final repolarization. Even in families without a history of LQTS, de novo mutations in cardiac ion channel genes may lead to sudden cardiac death in very young infants. (Circulation. 2001;104:1158-1164.)

Key Words: long-QT syndrome ■ arrhythmia ■ death, sudden ■ sodium ■ genes

Cardiac arrhythmias as the cause for syncope and sudden death in children and young adults are well known. A special role for the LQTS associated with sudden cardiac death in infants has been reported, and 3 recent reports also support the theory by identifying cardiac ion channel gene mutations in infants with aborted or experienced sudden infant death syndrome (SIDS). SIDS is defined as a sudden death, unexpected by clinical history, of an otherwise healthy infant in whom a thorough postmortem examination fails to detect an adequate cause of death. After the decline in infectious diseases, SIDS is the leading cause of death in the postneonatal period. The pathogenesis of SIDS is multifactorial, but the cardiorespiratory system and the central nervous system play a major role. Investigation of the LQTS genes in infants with SIDS or infants with premature sudden death has not been conducted systematically so far because tissue samples often are not available. The genetic information obtained from such infants may provide us with new clues of the

1. The congenital long-QT syndrome (LQTS) is a familial disorder that is characterized by prolongation of the QT interval on the surface ECG and episodes of syncope and/or life-threatening cardiac arrhythmias, specifically of polymorphic ventricular tachycardia (torsade de pointes, TdP) and sudden cardiac death. Six LQTS loci are known (LQTS1 to 6), and 5 cardiac ion channel genes have been identified. The genes at the LQTS1 (KCNQ1), LQTS2 (HERG), LQTS3 (KCNE1), and the LQTS6 (KCNE2) loci encode for potassium ion channel subunits, whereas the LQTS2 gene (SCN5A) encodes for an α-subunit of the cardiac sodium channel.

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pathogenic background in SIDS cases and will also address the question of the heritable factors as a cause for sudden infant death.

To date, more than 130 mutations in the LQTS genes have been identified; the majority of them are localized in the two cardiac potassium channels genes, KCNQ1 (LQTS1) and HERG (LQTS2). Mutations in the SCN5A gene (LQTS3) account for only 10% to 15% of all yet identified mutations in LQTS. Genotype-phenotype correlations suggest that patients with LQTS3 mutations have significantly more severe clinical events, including sudden cardiac death. In this article, we report a case of a sudden cardiac death in the third month of life caused by malignant tachycardia. We identified a de novo mutation in the SCN5A gene, and the electrophysiological data of the mutation suggest a possible new mechanism of affecting channel activity that leads to QT prolongation.

Methods

Genetic Analysis
Genomic DNA was isolated from venous EDTA blood of the infant and the family members by means of standard procedures. Genetic studies were performed in concordance with the recommendations of the ethics committee of the university and by the agreement of the parents. PCR primers were used as previously reported to amplify the ethics committee of the university and by the agreement of the parents. PCR primers were used as previously reported to amplify the entire coding regions of the 5 known LQTS genes. For both single-stranded conformational polymorphism (SSCP) and sequencing, a touchdown PCR was performed with the “hot start” technique.

Mutation Analysis With Fluorescent SSCP
SSCP analysis was performed according to the recommendations of the manufacturer (Amersham Pharmacia Biotech). Fluorescence-labeled PCR primers were used to amplify all coding exons and exon/intron boundaries of KCNQ1 HERG, SCN5A, KCNE1, and KCNE2. PCR products were analyzed with the A.L.F. Express DNA Sequencer (Amersham Pharmacia Biotech) connected to an external temperature control device; 3 µL of the PCR product was added to 1.5 µL of 50-bp/300-bp sizer and 4.5 µL of denaturing solution (formamide and 0.01% bromophenol blue). This mixture was incubated for 3 minutes at 98°C; 4 µL was applied to a 6% polyacrylamide gel (acrylamide/bisacrylamide, 99:1). Each exon was analyzed at 12°C and 18°C, and mutations were detected by differences in migration patterns compared with the wild type.

Mutation Analysis by Gene Sequencing
Sequence analysis was done with the use of a solid-phase template preparation procedure, which requires that one of the primers used for PCR is biotinylated. To prepare single-stranded DNA, streptavidine-covered paramagnetic particles (Dynabeads M-280, Dynal) were used as recommended by the supplier. The immobilized template was sequenced with nested fluorescence-labeled primers, following the instructions of the AutoRead T7 Sequencing Kit (Amersham Pharmacia Biotech). DNA electrophoresis and sequence analysis were performed on the A.L.F. DNA-sequence.

Confirmation of Paternity
In the index patient, paternity was proved by means of 10 highly polymorphic microsatellite markers (AmpFISTR Profiler Amplification Kit; Applied Biosystems). Fragment analysis was performed on an ABI Prism 310 Genetic Analyzer (Applied Biosystems) with internal length standards.

Site-Directed Mutagenesis
Mutant Na+ channel cDNA was prepared by mutagenesis on the pSP64T-H1 plasmid. Primers used for the site-directed mutagenesis were 5'–CAATGCCCTGTTGGCCCCATCCTCGGCATC–3' and 5'–CATGATGGACGGATGGGCCCCACCGAGG–3'. An Acc–I–Kpn fragment was subcloned into wild-type pSP64T-H1, and the mutant insert and ligation regions were completely analyzed by sequencing. The A1330P cDNA was then subcloned into the HindIII–Xbal sites of the expression vector pCGI (kindly provided by David Johns and Eduardo Marbán, Johns Hopkins University, Baltimore, Md) for bicistronic expression of the channel protein and GFP reporter in a Human Embryonic Kidney cell line (HEK 293).

Heterologous Expression of the Mutant Sodium Channel
To express mutant (A1330P) and wild-type (WT) hH1, HEK 293 cells were cotransfected with 2 µg of Na+ channel α-subunit cDNA (WT or mutant, respectively) and 2 µg hβ1b-subunit cDNA with the use of lipofectamine (Gibco BRL, Life Technologies). Transfected HEK 293 cells were cultured in minimum essential medium (Earles salts and l-glutamine) supplemented with nonessential amino acid solution, 10% fetal bovine serum, 100 IU/mL penicillin, and 100 µg/mL streptomycin in a 5% CO2 incubator at 37°C for 1 or 2 days. Only cells exhibiting green fluorescence were selected for further electrophysiological experiments.

Electrophysiology
Sodium currents were measured in the whole-cell configuration of the patch-clamp technique with the use of an Axopatch 200B amplifier (Axon Instruments) with 70% to 80% of the series resistance compensated and the following solutions (mM/L): bath (external) solution: NaCl 140, KCl 4.7, CaCl2 1.8, MgCl2 2.0, NaHCO3 4.3, Na,HPO4 1.4, glucose 11.0, HEPES 16.8, pH adjusted to 7.4 (25 mM/L NaOH); pipette (internal) solution: CsF 100, CsCl 40, EGTA 10, NaCl 10, MgCl2 2.0, HEPES 10, pH adjusted to 7.3 (25 mM/L NaOH).

Electrophysiological experiments were carried out at a room temperature of 21°C. Patch electrodes were pulled from borosilicate glass, heat-polished, and had a tip resistance of 2 to 3 MΩ when filled with pipette solution. Whole-cell currents were filtered at 5 kHz and digitized at 30 kHz. Voltage control, data acquisition, and analysis were accomplished by use of custom software.

The presence of a persistent inward sodium current was determined from the tetrodotoxin (Alomone Labs, Israel) (TTX)-sensitive current, by taking the average current amplitude during the last 50 ms of a 300-ms depolarization to −20 mV, relative to the holding current at −120 mV. Only those experiments were analyzed in which no shift in holding current was observed during wash-in of TTX.

The voltage dependence of the relative Na+ conductance activation, steady-state inactivation, and recovery from inactivation were determined by means of the voltage protocols as depicted in the figures. For all protocols, the holding potential was −120 mV and the pulse protocol cycle time was 5 seconds. Steady-state activation and inactivation curves were fit using the Boltzman equation:

$$ I/I_{max} = A/[1.0 + \exp((V_{1/2} - V)/k)] $$

where $V_{1/2}$ is the voltage for the half maximal (in)activation and the slope factor $k$. Recovery from inactivation was analyzed by fitting the data with a monoeXponential equation:

$$ I/I_{max} = A[1.0 - \exp(-t/t)], $$

where $t$ is the recovery time interval and $t_r$ is the time constant of recovery. The time course of inactivation was fitted by a 2-exponential equation:

$$ I/I_{max} = A[1.0 - \exp(-t/t)] + [A_2[1.0 - \exp(-t/t_2)]], $$

where $A$ and $A_2$ are fractions of fast and slow inactivation components and $t$ and $t_2$ are the time constants of fast and slow inactivating components, respectively. The results are expressed as mean±SEM; statistical analysis was done with the use of a Student’s $t$ test for comparison of 2 means.
Results
Clinical Presentation
The male infant was born after 39 weeks of gestation, with a body weight of 3910 g. He was the full-term product of a normal pregnancy, labor, and delivery. Apgar score was 9 at 5 and 10 minutes. The initial physical cardiovascular examination in the nursery was normal. The pulse was 130 to 160 bpm, blood pressure was 90/50 mm Hg, and body temperature was 36.9°C. At the first day of life, the newborn had recurrent episodes of sudden cyanosis and unconsciousness. Loud screams proceeded each episode. At the neonatal intensive care unit, ECG monitoring showed nonsustained runs of a ventricular tachycardia with a heart rate of \( \approx 300 \) bpm. The routine standard ECG taken at that time showed sinus rhythm, normal atrioventricular and intraventricular conduction time without QTc prolongation (Figure 1A); in subsequent ECG recordings, a prolonged QTc interval of 600 ms\(^{1/2}\) calculated according to Bazett’s formula\(^{16}\) (Figure 1B) was found that persisted. Furthermore, an apparent T-wave alternans and an increased QT dispersion was documented in the Holter monitoring (not shown). Conduction disturbances were not detected. There was no evidence for underlying heart disease. Physical examination including auditory evaluation was within regular limits. Serum levels of sodium, potassium, magnesium, and calcium were in the normal range.

The nonsustained tachycardias resembled typical TdP; therefore, oral propanolol therapy was initiated with a dosage of 5 mg/kg per day. Heart rates were 127 bpm on day 1 and 107 bpm under \( \beta \)-blocker therapy on day 5, respectively.

Within the next 7 weeks in the hospital, the infant remained asymptomatic in stable sinus rhythm; the minimal heart rate was 80 bpm. The tachycardia did not reappear, and the boy was discharged at 7 weeks with oral propanolol therapy (5 mg/kg per day) and a recommendation of home monitoring.

At 9 weeks the patient had a lethal tachyarrhythmia that was recorded by the home monitor (Figure 2). Cardiopulmonary resuscitation was performed by both parents and by medical emergency service but was finally unsuccessful. In the preregistered monitor recordings, recurrent bradycardias with a heart rate of 60 bpm were also documented (data not shown), which finally set up into TdP.

The clinical examination of the family (parents and brother) was completely inconspicuous. The history revealed no episode of tachycardias, syncopeces, or sudden unexpected deaths. The 12-lead ECG with measurement of the QT interval corrected for heart rate (QTc) was within normal range (Figure 3).
Genetic Analysis and Paternity Confirmation

All exons of the LQTS genes (KCNQ1, HERG, SCN5A, KCNE1, and KCNE2) were first screened by SSCP analysis. In exon 23 of the SCN5A gene, an abnormal migration pattern could be identified (Figure 4A). Subsequent sequencing of the corresponding amplicon identified a heterozygous nucleotide exchange at codon 1330 of the gene. The G-to-C transversion leads to an amino acid exchange from alanine to proline (Ala1330Pro) (Figure 4B). To exclude a possible polymorphism at this position, we tested the general population as determined by SSCP analysis in 150 unrelated healthy individuals (data not shown) in whom the abnormal pattern was absent.

Subsequent SSCP and sequence analysis of the parents' and the brother's DNA failed to identify the mutation (Figure 4A). Paternity analysis showed that the proband shared all alleles from each of his parents (data not shown) and thus confirmed paternity. The Essen-Möller value of paternity confirmation was determined to be 6.299 (paternity index of 5000), which corresponds to a 99.98% probability of paternity. Taken together, A1330P represented a de novo mutation in the infant.

Electrophysiological Data

First we tested whether the A1330P mutation also promotes a persistent inward sodium current, as observed for most LQTS3 mutations identified to date. WT (Figure 5A) and A1330P (Figure 5B) sodium currents (I_{Na}) were recorded at −20 mV, from a holding potential of −120 mV during control condition and in the presence of 30 μmol/L TTX. Neither WT nor A1330P I_{Na} showed a substantial persistent inward component at the end of a 300-ms depolarization (see TTX-sensitive current, inset in Figure 5). The I_{Na} amplitude at the end of the 300-ms depolarization was 0.38 ± 0.15% (n = 3) for WT channels and 0.13 ± 0.02% (n = 3) for A1330P mutant channels.

Next we investigated the activation and inactivation kinetics for both channel types. Figure 6 shows the current-voltage relation and the conductance-voltage and steady-state inactivation curves for WT and mutant channels. For both channel types, the threshold of activation was −60 mV and maximum peak inward current was observed at −25 mV (Figure 6A, B). There was no statistical difference between peak current amplitude of WT and the mutant at any voltage.

Figure 6C shows that the potential for half-maximal activation and slope factor were similar for WT and A1330P.
mutant channels (WT: $V_{1/2}$ = $-42.8 \pm 2.9$ mV, $k$ = $6.8 \pm 0.8$ (n=7); A1330P: $V_{1/2}$ = $-44.3 \pm 1.5$ mV ($P$ = 0.66), $k$ = $7.3 \pm 0.5$ ($P$ = 0.57), (n=7)). In contrast, the $V_{1/2}$ for inactivation of A1330P mutant channels was significantly shifted to more positive potentials compared with WT channels, from $-298.1 \pm 1.9$ mV (WT, n=7) to $-289.8 \pm 2.2$ mV (n=7, $P$, 0.05). The slope factors, $k$, were similar: $k$ = $5.8 \pm 0.3$ and $6.1 \pm 0.13$ for WT and A1330P, respectively. The positive shift in steady-state inactivation produced a greater overlap of activation and inactivation relations, the so-called “window current.” This is illustrated in an enlargement of the window region (see inset Figure 6D). This region for WT channels ranged from $-80$ mV to $-60$ mV, with a maximum of 2%, whereas that of A1330P mutant channels ranged between $-80$ mV and $-50$ mV, with a maximum of 4%.

To compare the time course of inactivation for both channel types, current decay was fitted with a double exponential function. Figure 7 shows that the time constants of both fast and slow inactivation were found to be slower in the A1330P mutant, although only significant at voltages positive to $-20$ mV, for example, WT: $\tau_f$ = $0.7 \pm 0.1$, $\tau_s$ = $4.9 \pm 0.8$; A1330P: $\tau_f$ = $1.1 \pm 0.1$, $\tau_s$ = $8.5 \pm 0.6$ ($P$ < 0.05) at +10 mV (Figure 7).

We also investigated the recovery from inactivation by using a 2-pulse protocol. Figure 8 shows the fraction of channels that had recovered from inactivation after various time intervals at $-120$ mV. A1330P mutant showed significantly faster recovery from WT channels (WT: $\tau$ = $17.2 \pm 2.0$ ms versus $\tau$ = $10.0 \pm 1.7$ ms for A1330P, $P$ < 0.05).

**Discussion**

In this report, we describe the sudden cardiac death of an infant at 9 weeks of age who died of documented TdP degenerating into ventricular fibrillation. The clinical diagnosis of LQTS was already made on day 2 after birth because of a prolonged QTc of 600 ms and recurrent TdP tachycardias for which the boy was immediately treated with oral propranolol. In addition, the infant had a number of other ECG characteristics that have been associated with SCN5A-linked forms of congenital LQTS, including delayed T-waves at rest after a long isoelectric ST-segment, exercise-induced QT interval shortening and TdP during sleep concomitant with bradycardia.\textsuperscript{17–19}
In most newborns and infants who had ventricular tachycardia with poor outcome, preexisting clinical or subclinical heart disease has been identified. Besides conditions such as myocarditis, cardiomyopathy, and congenital heart disease, the LQTS has also been described as the underlying cause for death. The majority of cardiac deaths caused by LQTS still occur in teenagers and adults. Deaths during the first months of life are uncommon. Our case demonstrates such a rare form as a cause for sudden infant death.

We identified a missense mutation (A1330P) in the DIIIIS4-S5 region of the human cardiac sodium channel α-subunit gene SCN5A. The mutation causes a substitution from alanine to proline, which is well recognized for its ability to disrupt protein secondary structure (particularly within α-helices). Unlike other amino acids, its side chain is covalently bound to the carbon, generating a rigid ring. At present, 16 SCN5A mutations have been identified and linked to the LQTS. Of these, 7 mutations (N1325S, ΔKPQ, R1623Q, R1644H, E1784K, D1790G and 1795insD) have been characterized in heterologous expression systems and are covalently bound to the carbon, generating a rigid ring. 23 At present, 16 SCN5A mutations have been identified and linked to the LQTS. Of these, 7 mutations (N1325S, ΔKPQ, R1623Q, R1644H, E1784K, D1790G and 1795insD) have been characterized in heterologous expression systems and were all found to cause a late component of sodium current by multiple mechanisms involving the inactivation process. It has been predicted that such a sustained sodium current during plateau phase of the action potential will prolong repolarization and thus accounts for the long QT interval. For the D1790G LQTS3 mutation, the mechanism of QT prolongation is debated.33 Besides a persistently inward sodium current, it displays a negative shift in voltage dependence of inactivation and may prolong the action potential through a calcium-dependent mechanism.

Thus, together with its localization in the DIIIIS4-S5 domain close to the proposed docking site mediating inactivation, one would expect the A1330P mutant to have similar alterations in electrophysiological properties as previously published LQTS3 mutants. Nevertheless, the A1330P mutant did not exhibit a detectable persistent inward current. Instead we observed an 8.3-mV shift in the voltage dependence of steady-state inactivation toward more positive values, whereas voltage dependence of activation was unaffected. This shift in inactivation predicts an increased channel availability at the resting membrane potential of ventricular cells but more importantly, an increase in the amplitude and voltage range of the so-called sodium “window current,” that is, the maintained inward sodium current caused by an overlap in activation and inactivation relations. Besides, we found alterations in the inactivation kinetics and the recovery from inactivation. Mutant channels exhibited a significantly slowed rate of current inactivation at potentials positive to −20 mV, whereas recovery from inactivation (at −120 mV) was accelerated. These results suggest that the A1330P mutation prolongs ventricular repolarization by an increase in sodium current during the plateau of the action potential caused by a slowing of the rate of inactivation and by an increase in the window sodium current during the final phase of repolarization. In summary, the A1330P mutant is distinguished from previous LQTS3 mutants by the absence of a persistent inward current at depolarized potentials and a positive shift in the voltage dependence of inactivation. (so far, shifts in inactivation reported for LQTS3 mutants were negatively directed).

Zareba and coworkers showed that patients with mutations in the SCN5A gene are associated with more lethal cardiac events compared with the overall number of cardiac events (20%) than patients with mutations in the LQTS1 and LQTS2 gene (4%). At the age of 40 years, mortality rates did not appear to be different in all 3 groups. The first death that occurred in this LQTS3 group was documented at the age of 8 years. Before this age, only LQTS1 mutation carriers had lethal events. In the present case, sudden arrhythmogenic death occurred at the age of 9 weeks, which raises the possibility that deaths associated with SCN5A mutations may be more frequent than recently estimated by Zareba and coworkers because of the exclusion for premature death of those cases from follow-up studies. This might lead to underestimation of the real incidence of LQTS3 patients in this age group. Early diagnosis and treatment of infants with LQTS is important because sudden cardiac death is more likely to occur as the initial and final event in children than in adults. Genetic analysis of the LQTS genes in neonates is therefore recommended in those cases in whom the suspicion of LQTS is made by family history or ECG findings including QTc at birth >500 ms or prenatal sinus bradycardia. Sporadic cases escape early diagnosis and treatment until the infant has the first symptoms or is discovered by other medical investigations by chance.

In summary, we identified a novel de novo mutation in the SCN5A gene in an infant with cardiac death in the third month of life. The electrophysiological data suggest a possible new

**Figure 7.** Fast and slow time constants of current decay as acquired by fitting current decay at each potential with biexponential function. Asterisk indicates significance (P<0.05); solid symbols, A1330P; and open symbols, WT.

**Figure 8.** Time course of recovery from activation with 2-pulse protocol shown in inset.
mechanism of affecting channel activity that leads to QT prolongation. Thus, the clinical presentation and the genetic and electrophysiological findings make this mutant very likely to cause the disease. Even in families without a history of LQTS, de novo mutations in cardiac ion channel genes may cause sudden infant death and, in extrapolation, the role of sporadic cases in SIDS must be further evaluated.

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