

Brief Report

A MOLECULAR LINK BETWEEN THE SUDDEN INFANT DEATH SYNDROME AND THE LONG-QT SYNDROME

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THE sudden infant death syndrome (SIDS) remains the leading cause of death in the first year of life and has a devastating impact on the affected families.¹⁻⁴ Despite the fact that there have been many hypotheses,^{3,4} the cause or causes of SIDS are still uncertain; as a consequence, the only preventive measure recommended is to avoid having infants sleep in a prone position.⁵

In 1998, we reported the results of a 19-year prospective study of more than 34,000 infants who underwent electrocardiography on the third or fourth day of life.⁶ We tested the hypothesis^{7,8} that the congenital long-QT syndrome accounts for a portion of the cases of SIDS.^{9,10} We found that 50 percent of the infants who died of SIDS had a prolonged QT interval corrected for heart rate (QTc) and that the presence of a prolonged QTc (>440 msec) in the first week of life increased the risk of SIDS by a factor of 41.⁶ This finding had implications with respect to the potential value of neonatal electrocardiographic screening.

Among the hypotheses that we had advanced to explain the origin of a prolonged QT interval in in-

fant, its relation to the increased risk of sudden death, and the fact that the parents of these infants had apparently normal electrocardiograms, two were testable. The first was that a spontaneous mutation occurs in one of the genes responsible for the long-QT syndrome,¹¹ and the second was that these infants are affected by a long-QT syndrome with a low penetrance.¹²

In this report we describe an infant who nearly died of SIDS, whose parents had normal QT intervals, and in whom the long-QT syndrome was diagnosed and a spontaneous mutation on the cardiac sodium-channel gene (*SCN5A*) was identified. Neonatal electrocardiographic screening would have made possible early identification of the prolonged long-QT interval and preventive treatment of this infant. Our findings in this single case report prove the validity of our first hypothesis and provide evidence of a link between the long-QT syndrome and SIDS. Our findings also demonstrate that spontaneous mutations in long-QT syndrome genes may manifest as and be indistinguishable from classic cases of near-SIDS or of SIDS itself.

CASE REPORT

On October 19, 1995, the parents of a 44-day-old infant who had a completely normal clinical history found him cyanotic, apneic, and pulseless. They rushed him to the emergency room of the local hospital, where an electrocardiogram showed ventricular fibrillation (Fig. 1A). Multiple DC shocks and mechanical ventilation were needed to restore sinus rhythm, and a marked prolongation of the QT interval was documented (QTc, 648 msec) (Fig. 1B). The plasma electrolyte levels were normal. Torsade de pointes recurred several times, often degenerating into ventricular fibrillation. The long-QT syndrome was diagnosed, treatment with propranolol (4 mg per kilogram of body weight) and mexiletine (10 mg per kilogram) was begun, and there were no recurrences of arrhythmias. At nearly five years of age, the child remains free of symptoms, and there have been no neurologic sequelae. At the time of the last follow-up examination at our institution, when he was three years old, the QT interval was still prolonged, but less severely so (QTc, 510 msec) (Fig. 1C). The child had no family history of the long-QT syndrome or SIDS, and the QT intervals of both his parents were within normal limits: his mother's QTc was 380 msec, and his father's QTc was 425 msec.

METHODS

Molecular Screening

Genomic DNA was extracted from peripheral-blood lymphocytes from the child and his parents with the use of standard techniques.¹³ Screening for mutations was performed in all genes known to be related to the long-QT syndrome with the use of specific oligonucleotide primer pairs.^{14,15} Single-strand conformational polymorphism analysis¹⁶ was used as a preliminary screening technique. Samples with mobility shifts were sequenced directly or were subcloned into a pBlueScript Sk- vector (Stratagene, La Jolla, Calif.) and then sequenced. Multiple sequences were compared with use of computer software (GCG Wisconsin Sequence Analysis Package, version 8.1, Genetics Computers Group, Palo Alto, Calif.).

Gene-Expression Studies

The normal, or wild-type, human-heart sodium-channel clone (hH1a) that we used has been described previously.^{17,18} The SIDS-

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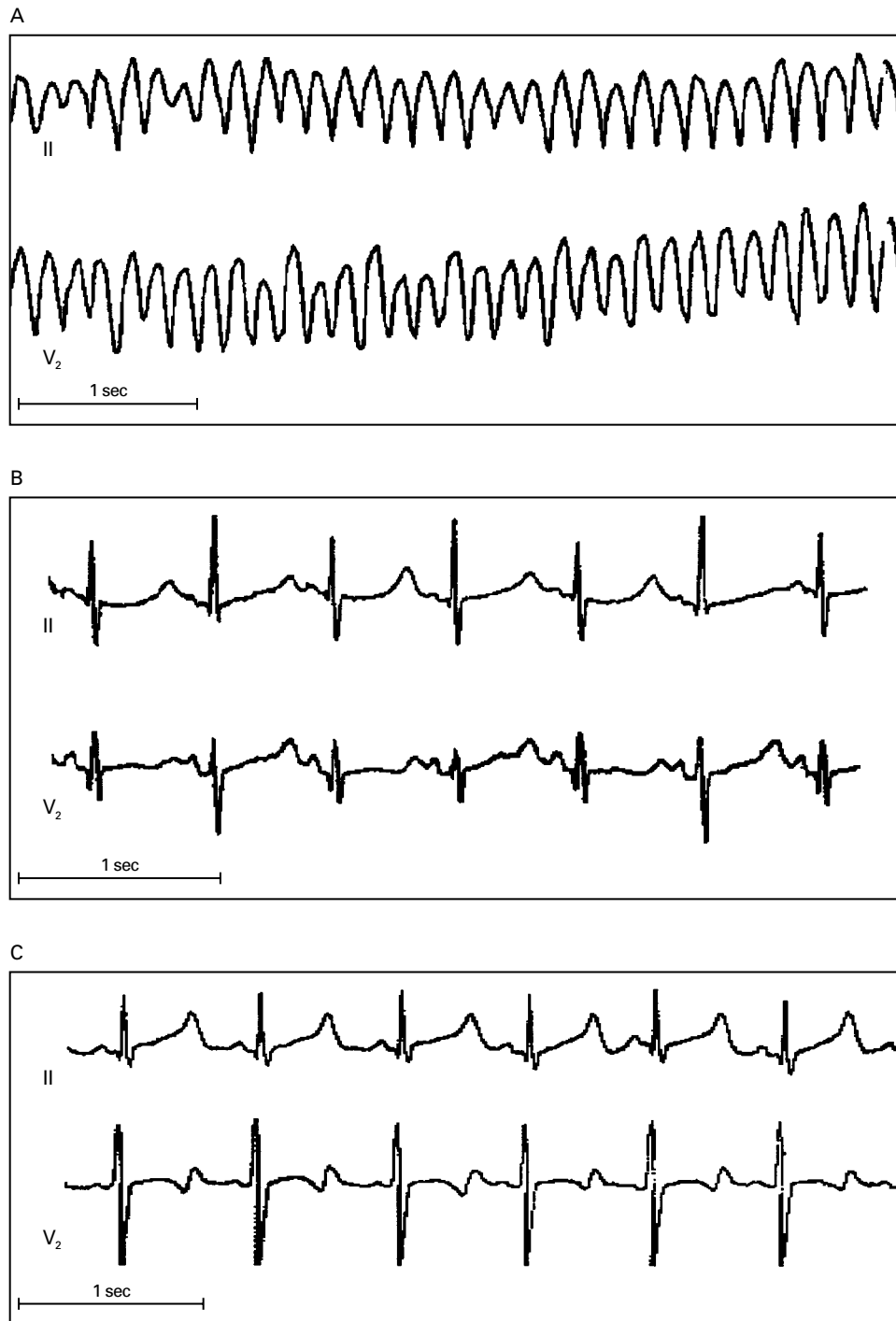


Figure 1. Electrocardiograms at the Time of Admission to the Hospital (Panel A), after the Restoration of Sinus Rhythm (Panel B), and at the Time of the Last Follow-up Visit (Panel C).

At hospital admission, the 44-day-old infant had ventricular fibrillation (Panel A). After the restoration of sinus rhythm, the corrected QT interval was found to be prolonged (648 msec) (Panel B). At the time of the last follow-up visit at the age of three years, the child's corrected QT interval, albeit still prolonged, was shorter (510 msec), possibly as a result of continued treatment with propranolol and mexiletine (Panel C).

LQTS mutation consisted of the substitution of AAC for TCC at positions 2971 to 2972 and was introduced into the wild-type construct by site-directed polymerase-chain-reaction mutagenesis¹⁹ and verified by sequencing. In vitro transcription of coding RNA, heterologous expression of the transcripts in xenopus oocytes, and electrophysiologic measurements were performed at room temperature, as described previously.¹⁷ We measured the amplitude of the late sodium current 300 msec after the beginning of the test pulse. Measurements made at the 300-msec isochrone are representative of the amplitude expected during the plateau (phase 2) of the ventricular action potential. We measured the late current as the current that was tetrodotoxin-sensitive and expressed it as the percentage of the peak current at the same voltage.

RESULTS

Molecular Screening

The substitution of two nucleotides (AAC for TCC) at the same codon (positions 2971 to 2972) was found in exon 16 of the coding sequence of *SCN5A* (Fig. 2) in genomic DNA from the child. These changes lead to the substitution of a single amino acid — asparagine (N) replaces serine (S) — at codon 941 (S941N). The AA portion of nucleotide 941 lies in the intracellular loop between the second and third transmembrane domains of *SCN5A*.¹⁴ This area is highly conserved in different species. The mutation was not found in 400 chromosomes obtained from 200 reference subjects.

Molecular screening of genomic DNA from the child's parents showed that neither had the S941N mutation. A set of highly polymorphic microsatellite markers was used to confirm paternity, thus demonstrating that the child had a spontaneous mutation in a gene for the long-QT syndrome.

Gene-Expression Studies

When expressed in frog oocytes, the S941N mutation in *SCN5A* caused a gradual increase in the

amplitude of the late sodium current (from 179 percent at -20 mV to 249 percent at 20 mV) (Fig. 3). At a voltage of 10 mV, which typically is present at the end of phase 2 of the ventricular action potential, the peak current was $33 \mu\text{A}$ and the conductance of the mutant late sodium channels was increased by a mean factor of 2.4 (237 percent) as compared with that of the wild-type channels.

DISCUSSION

The findings in this case report provide evidence that a life-threatening event in infancy, with features that meet all the criteria for SIDS or for near-SIDS, may be due to a spontaneous mutation in genes for the long-QT syndrome, which is therefore not present in the parents of the infant and which leads to sudden death as a result of ventricular fibrillation. Our findings also provide evidence of one of the mechanisms involved in SIDS, have implications with respect to the difficult issue of widespread electrocardiographic screening of neonates, and indicate that at least this subtype can be diagnosed and prevented.

This case has all the classic features of near-SIDS. Before the episode, the infant had appeared to be in perfect health. His age at the time of the episode — 7 weeks — is within the age range of 5 to 12 weeks during which the incidence of SIDS peaks.² The parents found him cyanotic, apneic, and pulseless and rushed him to the hospital while attempting cardiopulmonary resuscitation: this is the same sequence of events described by the parents of infants who have died of SIDS and of those who have nearly died. Ventricular fibrillation was documented in the emergency room; this point is important given the frequent statements that ventricular arrhythmias have

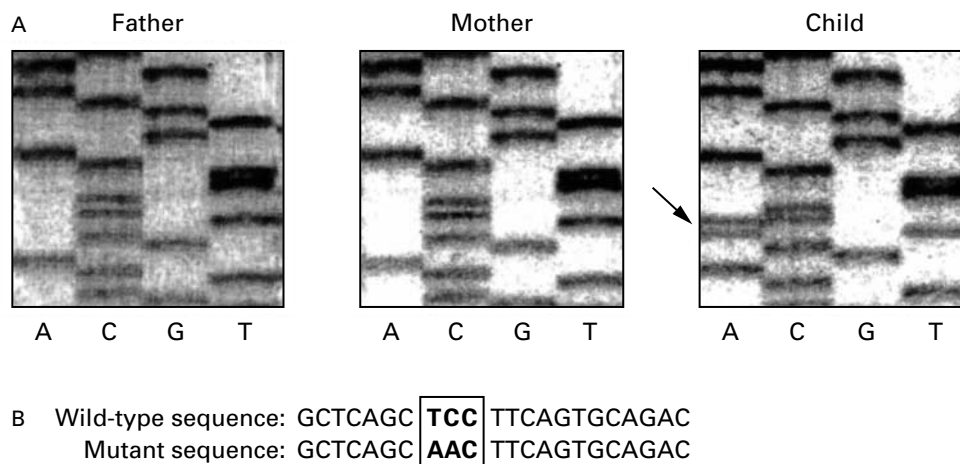


Figure 2. Results of Molecular Screening.

Analysis of DNA sequences of exon 16 of the *SCN5A* gene showed the presence of two abnormal bands indicating a heterozygous mutation in genomic DNA from the child (arrow), but not from his parents (Panel A). Panel B shows the wild-type sequence and the mutant sequence in which AAC is substituted for TCC.

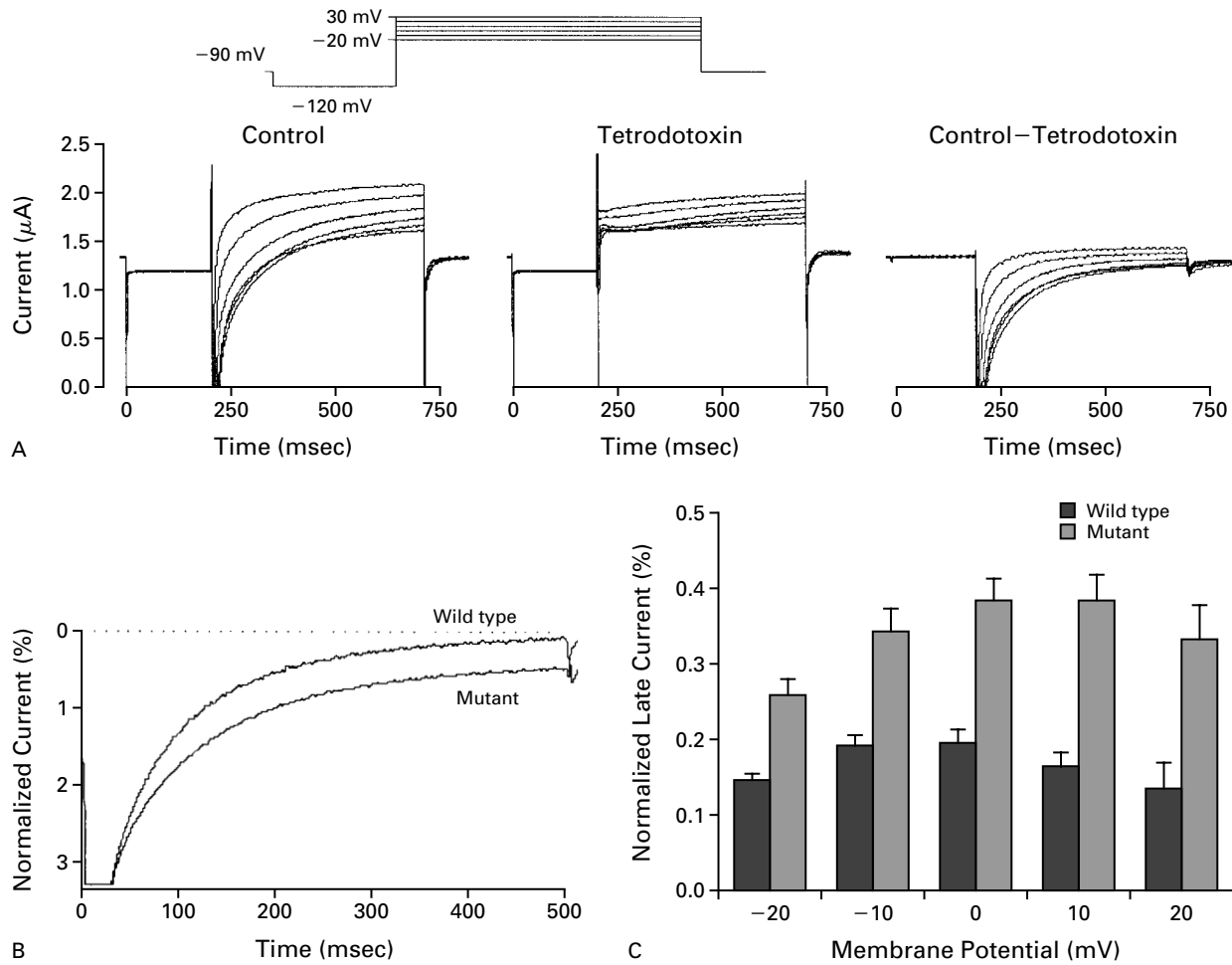


Figure 3. Effect of the S941N Mutation in *SCN5A* on Depolarization.

By increasing the amplitude of the late sodium current during depolarization, the S941N mutation in *SCN5A* contributes to the prolongation of the action potential observed in this infant, who had near-SIDS. In Panel A, a human-heart sodium-channel clone with the S941N mutation in *SCN5A* was introduced and expressed in frog oocytes, and the sodium-channel currents in the oocytes were recorded with a two-electrode voltage clamp. Cells were held at a membrane potential of -90 mV before the experiment and then exposed to a voltage of -120 mV to recruit all sodium channels available. The membrane potential was then sequentially increased to $-20, -10, 0, 10, 20,$ and 30 mV and then decreased to -90 mV , as shown in the upper part of the panel. In the lower part of the panel, to differentiate the late sodium current affected by the mutation from currents endogenous to the oocyte, recordings were made before (control) and after the addition of $50\text{ }\mu\text{M}$ tetrodotoxin, a highly specific sodium-current blocker. The recordings obtained after the addition of tetrodotoxin were then digitally subtracted from the control recordings to determine how much of the late sodium current was blocked by tetrodotoxin during each pulse. Panel B shows representative wild-type and mutant late sodium currents expressed as the percentage of the maximal current that was tetrodotoxin-sensitive and recorded at 0 mV . Panel C shows the relative amplitudes of the wild-type and mutant late sodium currents measured at 300 msec for membrane potentials representative of the plateau (phase 2) of the ventricular action potential. At each membrane potential, the difference in amplitudes between the two currents was significant ($P < 0.001$). Values are means (\pm SD) of seven experiments.

not been recorded in infants at risk for SIDS. Had the infant died — an outcome that was almost a certainty in the absence of cardioversion — the absence of an electrocardiogram and the normal QT intervals of both parents would have eliminated suspicion of the long-QT syndrome and would have prompted a diagnosis of SIDS.

The finding, after restoration of sinus rhythm, that the QT interval was greatly prolonged led to the diagnosis of the long-QT syndrome and to the institution of a therapy that proved to be effective, since the patient had no documented or symptomatic recurrences of arrhythmia. The long-QT syndrome may become apparent in the first few months of life²⁰ and

can be diagnosed at birth, usually on the basis of a finding of bradycardia resulting from a 2:1 atrioventricular block²¹ or of tachyarrhythmias; commonly, one parent has a prolonged QT interval. In our patient, both parents had normal QT intervals; thus, the diagnosis of the long-QT syndrome rested exclusively on the availability of the electrocardiographic findings.

Molecular screening of the patient and his parents provided the key to understanding how infants whose parents are unaffected die suddenly of the long-QT syndrome. The absence of the mutation in both parents and confirmation of paternity were essential to establish that the infant had a spontaneous mutation of a gene for the long-QT syndrome. All such genes identified to date encode ion channels involved in the control of ventricular repolarization.¹¹ The mutation found in this infant is in *SCN5A*, the cardiac sodium-channel gene responsible for the LQT3 subtype of the long-QT syndrome¹¹ and for the Brugada syndrome,²² another potential cause of sudden death in infancy.²³ Interestingly, patients with the subtype LQT3 have a further prolongation of the QT interval at night²⁴ and are particularly likely to die while at rest or in their sleep²⁵ and during their first arrhythmic episode.²⁶ The administration of sodium-channel blockers such as mexiletine shortens the QT interval in these patients²⁷ and may prevent the arrhythmias.²⁸

Dumaine et al.²⁹ showed that R1644H, another mutation known to cause the LQT3 subtype, increased the amplitude of the late sodium current by a factor of 2.3 under experimental conditions that were identical to ours. The increase in the amplitude of the late sodium current induced by the S941N mutation is therefore similar to that observed for other mutations known to give rise to the LQT3 subtype and is likely to be the basis for the prolongation of the QT interval observed in our patient. This late sodium current contributes prominently to the maintenance of phase 2 of the ventricular action potential, and thus, to its prolongation.³⁰ This finding links this case of near-SIDS to a defect in an ion channel associated with the long-QT syndrome.

The postmortem examination of patients who have died of SIDS and of those who have died of the long-QT syndrome usually fails to establish an abnormality sufficient to cause death. Recent data¹⁰ indicate that 14 percent of all patients with the long-QT syndrome die during their first episode of arrhythmia and that 30 percent of these deaths occur during the first year of life. If these patients had not been identified as having a family history of the long-QT syndrome, death would have been attributed to SIDS.

The case of our patient is unique because a molecular diagnosis of the long-QT syndrome was made in a patient who had all the key features of SIDS. How many similar cases go unrecognized? Maron et

al.³¹ described an infant who also presented at six weeks of age after nearly dying of SIDS, who had a markedly prolonged QTc (570 msec), who underwent defibrillation, and who led an active life without therapy despite the persistence of borderline prolongation of the QTc (460 msec) until she died in her sleep at the age of 12. This was apparently a case of the long-QT syndrome — possibly subtype LQT3, given her ability to participate in sports and the fact that she died during sleep.²⁵ It is important — and consistent with the occurrence of a spontaneous mutation — that all 18 of her first-degree relatives had a normal QT interval.

Data from almost 1000 families with a history of the long-QT syndrome indicate that treatment with beta-blockers reduces the mortality rate below 3 percent.¹⁰ This information is relevant to the prevention of SIDS in newborns with a prolonged QT interval, since it may provide a valid therapy in patients identified by neonatal screening as being at risk.

SIDS is multifactorial; the long-QT syndrome can account for only a fraction of the cases, and precise quantification of this fraction remains difficult despite the data obtained from our large epidemiologic study.⁶ The practical importance of this concept lies in the fact that most deaths due to the long-QT syndrome can be prevented.^{9,10} This implies that if the infants at risk were identified early on the basis of a consistently prolonged QT interval, preventive therapy could be instituted for a few months. Normalization of the QT interval during development would allow the withdrawal of therapy in the unavoidably large number of false positives while therapy might continue to protect the truly affected infants.

This case raises disquieting issues. Prompt defibrillation and restoration of sinus rhythm in our patient allowed the diagnosis of the long-QT syndrome to be made and lifesaving therapy to be instituted. The infant, however, could have died or could have suffered irreversible brain damage as a result of prolonged cardiac arrest; in the latter case, electrocardiography would still have led to the diagnosis. Undeniably, neonatal electrocardiography would have led to a much earlier diagnosis and institution of therapy and almost certainly would have prevented the life-threatening episode. Much of the current controversy surrounding neonatal electrocardiographic screening concerns the high number of false positives. This number could be decreased by postponing the screening until the second or third week of life. What has not been considered in the discussion is the emotional impact and the medicolegal consequences of cases such as the present one, in which diagnosis would be easy and treatment lifesaving. Myerburg³² has correctly stated that whether the cost of saving a young life exceeds the economic inefficiency of the screening tool will need to be determined by society as a whole.

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