Recurrent Third-Trimester Fetal Loss and Maternal Mosaicism for Long-QT Syndrome

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Background—The importance of germ-line mosaicism in genetic disease is probably underestimated, even though recent studies indicate that it may be involved in 10% to 20% of apparently de novo cases of several dominantly inherited genetic diseases.

Methods and Results—We describe here a case of repeated germ-line transmission of a severe form of long-QT syndrome (LQTS) from an asymptomatic mother with mosaicism for a mutation in the cardiac sodium channel, SCN5A. A male infant was diagnosed with ventricular arrhythmias and cardiac decompensation in utero at 28 weeks and with LQTS after birth, ultimately requiring cardiac transplantation for control of ventricular tachycardia. The mother had no ECG abnormalities, but her only previous pregnancy had ended in stillbirth with evidence of cardiac decompensation at 7 months’ gestation. A third pregnancy also ended in stillbirth at 7 months, again with nonimmune fetal hydrops. The surviving infant was found to have a heterozygous mutation in SCN5A (R1623Q), previously reported as a de novo mutation causing neonatal ventricular arrhythmia and LQTS. Initial studies of the mother detected no genetic abnormality, but a sensitive restriction enzyme–based assay identified a small (8% to 10%) percentage of cells harboring the mutation in her blood, skin, and buccal mucosa. Cord blood from the third fetus also harbored the mutant allele, suggesting that all 3 cases of late-term fetal distress resulted from germ-line transfer of the LQTS-associated mutation.

Conclusions—Recurrent late-term fetal loss or sudden infant death can result from unsuspected parental mosaicism for LQTS-associated mutations, with important implications for genetic counseling.

Key Words: long-QT syndrome • genetics • genes

Inherited forms of the long-QT syndrome (LQTS) have been associated with >200 different mutations in 7 genes encoding cardiac ion channels, their regulatory subunits, and a membrane anchoring protein.1,2 The vast majority of these mutations are dominant point mutations (Romano-Ward syndrome) that result in amino acid substitutions or non-sense codons. Current molecular genetic screening protocols can identify potentially causative mutations in approximately two thirds to three fourths of familial cases.3,4

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Recently, case reports and an epidemiological study have implicated LQTS in sudden unexplained infant death (sudden infant death syndrome [SIDS]).5–7 but genetic testing for LQTS is not widely available and is infrequently performed in cases of perinatal death. Hence, the extent of the contribution of LQTS to sudden infant death remains unknown.

When parents are clinically normal, a genetic basis for sudden death may not be suspected but could arise either from de novo mutation in the fetus or through parental germ-line transmission of a mutation. This latter phenomenon is surprisingly common and may underlie up to 30% of what otherwise appear to be de novo cases of dominantly inherited genetic disorders.8–11 Here, we describe the case of a clinically unaffected mother with somatic mosaicism for a known pathogenic mutation in the cardiac sodium channel, SCN5A, who transmitted a severe form of LQTS to multiple successive pregnancies. These findings have significant implications for the evaluation of causes of late-term fetal distress and demise, and for genetic counseling of apparently de novo cases of LQTS.

Methods

Clinical Subjects
A 1340-g black male infant was delivered by emergency cesarean section at 32 weeks’ gestation because of fetal arrhythmias and hydrops. Ventricular arrhythmias were initially diagnosed in utero at 28 weeks of gestation. An initial obstetrical ultrasound at 28 weeks...
revealed no gross abnormalities, but a subsequent fetal echocardiogram identified frequent bursts of atrial and ventricular tachycardia and episodes of 2:1 AV block. β-Blockers were ineffective, and a trial of amiodarone resulted in marked worsening of arrhythmias, with development of myocardial dysfunction and fetal hydrops necessitating emergency caesarian section. An ECG demonstrated AV conduction abnormalities and a QTc interval of 550 milliseconds (Figure 1A). After delivery, the newborn continued to have drug-resistant ventricular tachyarrhythmias, frequent episodes of ventricular flutter, and torsade de pointes (Figure 1B). A chest x-ray was consistent with hyaline membrane disease and mild cardiomegaly.

Tachyarrhythmias were partially responsive to lidocaine infusion but resisted a variety of other antiarrhythmic interventions, including epicardial pacing, esmolol infusion, bicarbonate, magnesium, calcium, mexiletine, flecaïnine, and left sympathetic ganglionectomy. Ultimately, the infant received orthotopic heart transplantation at 5.5 months of age. He is now almost 3 years of age and has had no further arrhythmias.

The infant’s mother was a 20-year-old black woman with no clinical evidence or family history of heart disease, cardiac arrhythmias, prematurity, syncope, cardiac arrest or sudden cardiac death, or autoimmune disorders. Her baseline ECG was normal (Figure 1C),
and her QTc interval did not lengthen abnormally during amiodarone infusion given for treatment of fetal arrhythmias (not shown). Her 1 previous pregnancy had ended in stillbirth at 28 weeks (Figure 1D); a report of the postmortem evaluation of the male fetus described marked cardiomegaly and a supernumerary digit but no other gross abnormalities. Prenatal care was started 10 weeks into her second pregnancy, and all prenatal laboratory studies were normal. The infant’s father was 19 years of age and in excellent health, but he declined to undergo genetic evaluation. The mother’s father and mother were 23 and 18, respectively, at the time of her birth.

During these investigations, the mother became pregnant for a third time by a second male partner. Regular prenatal examinations and monthly ultrasound studies revealed no abnormalities until the seventh month. At 28 weeks’ gestation, echocardiography revealed no fetal heartbeat, and a stillborn female infant was delivered by induction. Autopsy of this fetus revealed a severely enlarged but structurally normal heart and nonimmune hydrops fetalis, consistent with low-output cardiac failure; other organ systems were unremarkable. Cord blood was obtained for genetic analysis.

All subjects were recruited and evaluated in accordance with regulations set forth by the University of Miami Committee for the Protection of Human Subjects and under a human subjects research protocol approved by the University of Miami Institutional Review Board. Autopsy results on the stillborn fetuses and cord blood from the third fetus were provided with written consent of the mother.

**DNA Isolation**

Genomic DNA was obtained from lymphocytes, cultured skin fibroblasts, paraffin-embedded tissue, and buccal swab with a Puregene DNA Isolation Kit (Gentra Systems). DNA yields were determined spectrophotometrically.

**Single-Stranded Conformational Polymorphism Analysis**

The first 300 base pairs of exon 28 of SCN5A were amplified with polymerase chain reaction (PCR). 3 PCR was also performed on most of the exons from LQT1, LQT2, KCNE1, and KCNE2 with the use of published primers and conditions. 3,12 Single-stranded conformational polymorphism (SSCP) analysis was performed on a Bio-Rad Protein II xi Cell electrophoresis apparatus. Gels were made with or without glycerol using MDE Gel Solution (Cambrex BioScience) according to the manufacturer’s recommendations. Gels were developed with a silver stain kit from Bio-Rad according to their instructions.

**DNA Sequence Analysis**

PCR products to be sequenced were purified with a QIAquick PCR Purification Kit (Qiagen). Cycle sequencing reactions were performed by use of reagents from Applied Biosystems according to their recommendation. The products were resolved on an ABI Prism 3100 Genetic Analyzer and analyzed with ABI Sequence Analysis software. Hinfl (10 U/μL, New England Biolabs) digestions were done for 3 hours at 37°C in 20-μL reactions with up to 400 ng of PCR product and 10 U of enzyme. Digestion products were analyzed by electrophoresis in 2% agarose gels and staining with ethidium bromide. The intensity of bands was quantified with Sigmascan software (SPSS Science).

**Results**

**Mutation Analysis**

To identify the molecular abnormality in the index case, we conducted mutation screening of 5 cardiac ion channel genes, including KCNQ1, HERG, SCN5A, minK, and MiRP-1, covering ≈85% of the mutations known to be associated with LQTS.3 Analysis of the proband’s lymphocyte DNA revealed an unusual banding pattern of the first half of exon 28 of SCN5A on SSCP analysis. DNA sequencing of this fragment revealed that the infant was heterozygous for a previously reported mutation at nucleotide 486513,14 (Genbank NM_198056), with both a normal guanine residue and a mutant adenine residue (Figure 2A). This introduces a mutation in the second position of codon 1623, which changes a positively charged arginine to a neutral glutamine (R1623Q). No mutations were found in any of the other genes investigated.

**Detection of Maternal Mosaicism**

Initial SSCP analysis of the mother’s lymphocyte DNA gave an ambiguous result (not shown), but DNA sequencing suggested that she was homozygous for the wild-type allele (Figure 2B and 2E). To resolve this discrepancy, we took advantage of the fact that the R1623Q mutation destroys an
**Discussion**

In this case report, we describe the late-term loss or near loss of 3 successive pregnancies in a woman with somatic mosaicism for a lethal LQTS mutation. Although material for genetic studies was available only for 2 of the 3 infants, we provide strong evidence for germ-line transfer of the mutation. Genetic mosaicism results when a mutation occurs in a single cell of the developing embryo and is propagated only to its descendant cells in the adult. Mosaicism can affect either somatic or germ-line lineages or both, depending on the location and embryological stage at which the mutation occurs. It has been estimated that 10% to 20% of ostensibly de novo cases of retinoblastoma, hemophilia B, Duchenne muscular dystrophy, and hemophilia A are the result of parental mosaicism for these genetic disorders. Examples of germ-line mosaicism in asymptomatic parents have been documented in several autosomal dominant genetic diseases, including neurofibromatosis, osteogenesis imperfecta, and hypertrophic cardiomyopathy. The R1623Q mutation was first reported in an Asian female infant who, like our proband, presented perinatally with 2:1 AV block, lengthened QT interval, cardiac failure, and life-threatening ventricular arrhythmias. The child is now at least 8 years old and is free of arrhythmias on 600 mg/d mexiletine. Our patient is now almost 3 years old and is also free of arrhythmias after receiving an orthotopic heart transplant. A third, sporadic instance of this mutation has also been reported in an infant. The severe phenotype manifested by these infants is typical of a subset of LQTS patients who present in fetal and early neonatal life. A review of 22 case studies from the English language literature described a subset of LQTS patients with a severe neonatal-onset phenotype characterized by very long QTc intervals (mean, 665 milliseconds), 2:1 AV block, T-wave alternans, functional bradycardia, and a poor prognosis, with 9 of 22 dead in the first year of life and 50% mortality by 4 years of age. In comparison, a study of LQTS in a large pediatric collaborative study (mean age at diagnosis, 7 years) found a mean QTc of 520 milliseconds and a low rate of AV block (4%). A further striking feature of the perinatal LQTS phenotype was the infrequency of a family history of the disease (1 of 22, or 4.5%, compared with 60% in the collaborative study of pediatric LQTS). The important role of de novo mutations in perinatal LQTS is supported by the fact that disease-causing mutations at the same SCN5A residue (1623) have arisen independently at least 3 times and a number of other spontaneous mutations in LQTS-associated genes have been linked to lethal arrhythmia.

**HindIII restriction enzyme site (GANTC→AANTC).** As expected, the wild-type exon 28 PCR product was completely digested by HindIII, but more than half of the DNA from the index case remained undigested, corresponding to the presence of homoduplex mutant and heteroduplex strands lacking the restriction site (Figure 2C, lanes NC and PL). Analysis of the mother’s lymphocyte DNA also revealed a small amount of material resistant to complete digestion (Figure 2C, lanes ML, MF, and MB). When purified, amplified, and sequenced, this DNA was found to consist almost exclusively of the mutant allele (Figure 2D), consistent with maternal somatic mosaicism for the 

**mosaicism for the R1623Q mutation.**

We next used the HindIII assay to compare levels of mosaicism in several different maternal tissues (Figure 2C). Using a standard curve developed by serial dilution of proband DNA into normal DNA, we determined that the HindIII assay was sufficiently sensitive to permit detection of mutant DNA corresponding to ~4% heterozygous cells. Comparison of DNA from 3 different tissues suggested that mosaicism was greatest in circulating lymphocytes (~10%), less in buccal epithelium (8%), and least in skin fibroblasts (4%) (Figure 2C). These differences may reflect genuine but small variations in the proportion of mutation-bearing cells or alternatively variability in PCR amplification. A comparably low level of mosaicism in the heart would be consistent with the mother’s lack of symptoms or ECG findings, although this could not be directly confirmed.

The mother’s third pregnancy ended in stillbirth at 28 weeks. Analysis of fetal cord blood by HindIII digestion and sequencing revealed the presence of the mutant allele (Figure 2C, lane CB).

**Several lines of supportive evidence have emerged, including an ECG screening study of newborns, a case of a near-miss SIDS infant who carried an apparently de novo SCN5A mutation, and a case of a SIDS infant in whom postmortem molecular screening identified an apparently de novo mutation on KCNQ1. The latter study likely underestimates the true contribution of LQTS to unexplained infant death because even in clinically well-defined cases, mutations are not always identified.**

Our findings demonstrate that LQTS mutations, particularly those affecting the sodium channel encoded by SCN5A, confer a risk for late-term fetal loss and for infant sudden death.

In this case, the mother was found to harbor a small population of cells with the same SCN5A (R1623Q) mutation found in her children, indicating that she is a somatic mosaic for LQTS. Genetic mosaicism results when a mutation occurs in a single cell of the developing embryo and is propagated only to its descendant cells in the adult. Mosaicism can affect either somatic or germ-line lineages or both, depending on the location and embryological stage at which the mutation occurs. It has been estimated that 10% to 20% of ostensibly de novo cases of retinoblastoma, hemophilia B, Duchenne muscular dystrophy, and hemophilia A are the result of parental mosaicism for these genetic disorders. Examples of germ-line mosaicism in asymptomatic parents have been documented in several autosomal dominant genetic diseases, including neurofibromatosis, osteogenesis imperfecta, and hypertrophic cardiomyopathy.

The R1623Q mutation was first reported in an Asian female infant who, like our proband, presented perinatally with 2:1 AV block, lengthened QT interval, cardiac failure, and life-threatening ventricular arrhythmias. The child is now at least 8 years old and is free of arrhythmias on 600 mg/d mexiletine. Our patient is now almost 3 years old and is also free of arrhythmias after receiving an orthotopic heart transplant. A third, sporadic instance of this mutation has also been reported in an infant. The severe phenotype manifested by these infants is typical of a subset of LQTS patients who present in fetal and early neonatal life. A review of 22 case studies from the English language literature described a subset of LQTS patients with a severe neonatal-onset phenotype characterized by very long QTc intervals (mean, 665 milliseconds), 2:1 AV block, T-wave alternans, functional bradycardia, and a poor prognosis, with 9 of 22 dead in the first year of life and 50% mortality by 4 years of age. In comparison, a study of LQTS in a large pediatric collaborative study (mean age at diagnosis, 7 years) found a mean QTc of 520 milliseconds and a low rate of AV block (4%). A further striking feature of the perinatal LQTS phenotype was the infrequency of a family history of the disease (1 of 22, or 4.5%, compared with 60% in the collaborative study of pediatric LQTS). The important role of de novo mutations in perinatal LQTS is supported by the fact that disease-causing mutations at the same SCN5A residue (1623) have arisen independently at least 3 times and a number of other spontaneous mutations in LQTS-associated genes have been linked to lethal arrhythmia.

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**Notes:**

mias in the fetus and neonate. The poor prognosis of infants with neonatal LQTS undoubtedly also contributes to low heritability.

The evidence for SCN5A mutations as a cause of perinatal LQTS seems to conflict with earlier studies showing that patients with LQTS generally have clinical manifestations later in life than patients with mutations at other loci and that SCN5A mutation penetrance is strongly age dependent. However, the findings presented here demonstrate that certain SCN5A mutations can have an early, aggressive phenotype characterized by arrhythmias and death before birth or in early infancy. These mutations do not appear to overlap with those previously reported to cause disease later in life. Additional work is required to explain the mechanisms underlying these distinct clinical presentations.

The mutation detected in our patient lies in the S4 region of SCN5A domain IV, which participates in coupling of channel activation and inactivation. DNA sequence alignment of the human isoforms of this sodium channel shows that the 3 brain-type (1A, 2A, and 3A), the skeletal muscle (4A), and the cardiac (5A) isoforms are >90% identical in this region, suggesting that this part of the protein serves a general and essential function of the channel. The severity of disease associated with mutations in this portion of the channel further underscores its functional importance. In addition to 3 apparently de novo R1623Q mutations being associated with a severe, early-onset phenotype of LQTS (this report and apparently de novo further underscores its functional importance. In addition to 3 SCN5A mutations can have an early, aggressive phenotype characterized by arrhythmias and death before birth or in early infancy. These mutations do not appear to overlap with those previously reported to cause disease later in life. Additional work is required to explain the mechanisms underlying these distinct clinical presentations.

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The role of genetics in fetal demise is likely to be overlooked, particularly when parents have no evidence of disease. Dominant mutations such as those leading to LQTS, hypertrophic cardiomyopathy, and other disorders associated with sudden death are often assumed to have arisen de novo in the affected individual (see elsewhere for review). The possibility of genetic mosaicism might not be explored, partly because of technical difficulties in detection and partly because of a low level of suspicion when both parents are clinically normal. Nonetheless, the distinction between de novo mutation and parental germ-line mosaicism is critical because mosaicism confers a considerably higher risk of disease recurrence in subsequent pregnancies. The frequency of de novo or transmitted mosaic LQTS mutations in fetal demise or SIDS remains to be established. Our observation of repeated germ-line transmission of LQTS from a mother with unsuspected mosaicism implies that perinatal cardiac mosaicism, in addition to genetic counseling, should be considered after recurrent late-term fetal loss or infant death. Genetic disorders linked to cardiac sudden death may be an important focus for future genetic epidemiological studies of unexplained third-trimester pregnancy loss.

Acknowledgments

This work was supported by a collaborative grant from the Miami Heart Research Institute (N.H.B. and R.J.M.), a Grant-in-Aid from the Florida-Puerto Rico Affiliate of the American Heart Association (L.B.), and a generous contribution from the Dr John T. Macdonald Foundation for Medical Genetics. We are grateful to Drs Mark Keating and Igor Saplowski for providing control DNA samples, unpublished data, and technical suggestions throughout these experiments. We thank Dr Louis Elias for his review and comments on the manuscript.

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_Circulation_. 2004;109:3029-3034; originally published online June 7, 2004;
doi: 10.1161/01.CIR.0000130666.81539.9E
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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