Developmentally regulated *SCN5A* splice variant potentiates dysfunction of a novel mutation associated with severe fetal arrhythmia

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BACKGROUND Congenital long-QT syndrome (LQTS) may present during fetal development and can be life-threatening. The molecular mechanism for the unusual early onset of LQTS during fetal development is unknown.

OBJECTIVE We sought to elucidate the molecular basis for severe fetal LQTS presenting at 19 weeks' gestation, the earliest known presentation of this disease.

METHODS Fetal magnetocardiography was used to demonstrated torsades de pointes and a prolonged rate-corrected QT interval. In vitro electrophysiological studies were performed to determine functional consequences of a novel *SCN5A* mutation found in the fetus.

RESULTS The fetus presented with episodes of ventricular ectopy progressing to incessant ventricular tachycardia and hydrops fetalis. Genetic analysis disclosed a novel, de novo heterozygous mutation (L409P) and a homozygous common variant (R558 *in SCN5A*). In vitro electrophysiological studies demonstrated that the mutation in combination with R558 caused significant depolarized shifts in the voltage dependence of inactivation and activation, faster recovery from inactivation, and a 7-fold higher level of persistent current. When the mutation was engineered in a fetal-expressed *SCN5A* splice isoform, channel dysfunction was markedly potentiated. Also, R558 alone in the fetal splice isoform evoked a large persistent current, and hence both alleles were dysfunctional.

CONCLUSION We report the earliest confirmed diagnosis of symptomatic LQTS and present evidence that mutant cardiac sodium channel dysfunction is potentiated by a developmentally regulated alternative splicing event in *SCN5A*. Our findings provide a plausible mechanism for the unusual severity and early onset of cardiac arrhythmia in fetal LQTS.

KEYWORDS Arrhythmia; Sodium channel; *SCN5A*; Sudden death; Long-QT syndrome; Magnetocardiography; Alternative splicing

ABBREVIATIONS **AV** = atrioventricular; **LQTS** = long-QT syndrome; **TdP** = torsades de pointes; **WT** = wild type

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Introduction

Congenital long-QT syndrome (LQTS) refers to a group of disorders with the primary impairment of myocardial repolarization predisposing to life-threatening cardiac arrhythmias especially torsades de pointes (TdP) that are caused by genetic mutations in cardiac ion channels or channel-modulating proteins.[1] The disease is typically recognized in late childhood or early adolescence, but extreme cases may present during infancy or in the perinatal period.[2–5] Clinical signs suggestive of fetal LQTS include ventricular tachycardia, second-degree atrioventricular (AV) block, and, most commonly, sinus bradycardia,[6,7] but such findings may go undetected owing to the lack of routine electrocardiographic testing of fetuses. Evidence for Mendelian inheritance is not always apparent in cases of fetal LQTS because of de novo mutations or germ line mosaicism.[8,9] Certain *SCN5A* mutations, many of which are de novo,[2,4,5,10–14] present with earlier onset and more severe congenital arrhythmia syndromes than is typical for LQTS. The reason for greater severity and lethality of certain genetic variants during early life is unknown.

Here we report the clinical, electrocardiographic, and genetic diagnosis of LQTS in a fetus at 19 weeks' gestation presenting with ventricular tachycardia and severe hydrops fetalis. To our knowledge, this is the earliest gestational age at which a diagnosis of LQTS has been made after being

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suspected on the basis of clinical presentation. A novel, de novo *SCN5A* mutation combined with a common genetic variant was discovered in the proband, and we demonstrated a plausible molecular basis for arrhythmia presentation. Specifically, we determined that the mutation and common variant both conferred severe functional disturbances when expressed in the context of a cardiac sodium channel isoform generated by a developmentally regulated *SCN5A* alternative splicing event. Our findings implicate dysfunction of a fetal-expressed sodium channel splice isoform as a predisposition to intrauterine mortality in LQTS. These results may have relevance to perinatal and neonatal deaths in other clinical settings.

Methods

Testing for mosaicism

Parental DNA extracted from blood, saliva, and buccal swabs was examined by using direct sequence, restriction enzyme digest (*Eag*I, *Msp*I, or *Nci*II), and TaqMan allelic discrimination assay.

Measurement of cardiac *SCN5A* expression

De-identified, frozen, postmortem heart tissues from white American subjects were obtained from the Brain and Tissue Bank of the University of Maryland under an exemption for human subject research granted by the Vanderbilt University Institutional Review Board. Total RNA was isolated and used for quantitative real-time polymerase chain reaction by using TaqMan probes specific for *SCN5A* exon 6 or exon 6A to measure the relative levels of mRNA transcripts containing either of these alternate exons. Additional details of these experimental methods are provided in the online supplement.

Mutagenesis and heterologous expression of human cardiac sodium channel

Mutagenesis of recombinant human cardiac sodium channel ($Na_V1.5$) was performed as described previously,[5,15] except that a rare variant present in the original cDNA (glutamine-1027; GenBank accession number M77235)[16] was reverted to the common allele (arginine-1027). The common variant R558 was engineered in some constructs to match the genotype of the study subject. Wild-type (WT) or L409P mutant channel cDNA (0.5 μg) was transiently transfected into tsA201 cells by using FuGene 6 (Roche Diagnostics, Indianapolis, IN) combined with a plasmid encoding enhanced green fluorescent protein (IRES2-eGFP, 0.5 μg). Transiently transfected cells were incubated 48 hours at 37°C prior to electrophysiological measurements. Cells exhibiting green fluorescence were selected for patch-clamp recordings. A fetal $Na_V1.5$ cDNA was engineered by making the following amino acid substitutions encoded by the alternate exon 6 (designated exon 6A): V206T, S207T, N209F, I210V, K211D, L215V, and P234S.

In vitro electrophysiology

Sodium currents were recorded at room temperature by using the whole-cell patch-clamp technique as described previously,[5,15] with additional details provided in the online supplement. Results are presented as mean ± SEM. Unless otherwise noted, statistical comparisons were made by using an unpaired Student *t* test in reference to WT $Na_V1.5$. Statistical significance was assumed for $P < .05$.

Results

Identification of a novel *SCN5A* mutation in a fetus with TdP

A 29-year-old primiparous woman was referred for the evaluation of an irregular fetal heart rhythm at 19⅐ weeks' gestation (by the last menstrual period and an 11-week ultrasound). There was no family history of pregnancy loss, syncope, seizures, sudden cardiac death at any age, accidental death, or drowning. An initial fetal echocardiogram at 20 weeks' gestation disclosed normal cardiac anatomy with decreased ventricular function, mild tricuspid valve insufficiency, and a very small pericardial effusion. The atrial rate was regular at 130–160 beats/min, but the ventricular rate was variable. There were frequent premature ventricular contractions and couplets, and short (3–4 beats) runs of tachycardia (Figure 1A). During nonsustained tachycardia, the atrial rate was slower than the ventricular rate, leading to a presumptive diagnosis of ventricular tachycardia. There was no evidence of AV block. Maternal electrolytes were normal, and serum testing for maternal SSA/SSB antibodies, immunoglobulin G to cytomegalovirus, and *Toxoplasma gondii* was negative. The corrected QT intervals determined from 12-lead electrocardiogram recordings were in the normal range for the patient and fetus' father (424 and 383 ms, respectively). At 20⅐ weeks, a fetal magnetocardiogram revealed frequent short episodes of polymorphic ventricular tachycardia consistent with TdP and a corrected QT interval of 604 ms (Figures 1B and 1C). During 2 hours of data recording, AV block was not observed. An ultrasound on the same day showed interval accumulation of pleural fluid and ascites consistent with hydrops fetalis. The treatment of the fetal arrhythmia was discussed with the family; however, because of the dire clinical status, the parents elected not to pursue the treatment.

Echocardiography at 22 weeks' gestation revealed severe cardiac dysfunction, more frequent and more prolonged episodes of ventricular tachycardia (Figure 1D), and worsening hydrops fetalis. Although tachycardia cycle length was similar to that observed 2 weeks earlier (Figure 1A), the velocity of Doppler signals was extremely low, suggesting that the stroke volume was greatly decreased during tachycardia episodes. At this time, tachycardia episodes had a longer duration, and the intervals between tachycardia episodes were shorter (not shown), implicating increased tachycardia burden as a factor for the progression of cardiac dysfunction. On the basis of the extent of clinical deterioration, pregnancy was terminated at the request of the family. Postmortem genetic testing (Familion) of the fetus identified a novel heterozygous *SCN5A* transition mutation (T1226C), predicting a missense change in codon 409 from leucine to proline (designated *SCN5A*-L409P). No muta-