Published in final edited form as:

Heart Rhythm. 2012 April ; 9(4): 590–597. doi:10.1016/j.hrthm.2011.11.006.

Developmentally Regulated *SCN5A* Splice Variant Potentiates Dysfunction of a Novel Mutation Associated with Severe Fetal Arrhythmia

Lisa L. Murphy, B.S.^{1,*}, Anita J. Moon-Grady, M.D.^{2,*}, Bettina F. Cuneo, M.D.³, Ronald T. Wakai, Ph.D.⁴, Suhong Yu, M.S.⁴, Jennifer D. Kunic, B.S.⁵, D. Woodrow Benson, M.D., Ph.D.⁶, and Alfred L. George Jr., M.D.^{1,5}

¹Department of Pharmacology, Vanderbilt University, Nashville, TN

²Department of Pediatrics, University of California at San Francisco, San Francisco, CA

³The Heart Institute for Children, Hope Children's Hospital, Oak Lawn, IL

⁴Department of Medical Physics, University of Wisconsin-Madison, Madison WI

⁵Department of Medicine, Vanderbilt University, Nashville, TN

⁶Division of Cardiology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH

Abstract

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 demonstrate torsade 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 in *SCN5A* (L409P) and a homozygous common variant (R558). *In vitro* electrophysiological studies demonstrated that the mutation in combination with R558 caused significant depolarized shifts in voltage-dependence of inactivation and activation, faster recovery from inactivation and a 7-fold greater 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, hence both alleles were dysfunctional.

© 2011 The Heart Rhythm Society. Published by Elsevier Inc. All rights reserved.

Correspondence to: Alfred L. George, Jr., M.D., Division of Genetic Medicine, 529 Light Hall, Vanderbilt University, 2215 Garland Avenue, Nashville, TN 37232-0275, Phone: (615) 936-2660, Fax: (615) 936-2661, al.george@vanderbilt.edu.

*These two authors contributed equally

Conflicts of Interest: None

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

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

Introduction

Congenital long-QT syndrome (LQTS) refers to a group of disorders with primary impairment of myocardial repolarization predisposing to life-threatening cardiac arrhythmias especially torsade 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 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 death in other clinical settings.

Methods

Testing for mosaicism

Parental DNA extracted from blood, saliva and buccal swabs was examined by direct sequence, restriction enzyme digest (*EagI*, *MspI* or *NciII*) 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 real-time quantitative RT-PCR using TaqMan probes specific for *SCN5A* exon 6 or 6A to measure 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) were transiently transfected into tsA201 cells 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, P234S.

In vitro electrophysiology

Sodium currents were recorded at room temperature 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* < 0.05.

Results

Identification of a novel *SCN5A* mutation in a fetus with torsade de pointes

A 29-year-old primiparous woman was referred for evaluation of an irregular fetal heart rhythm at 19 ½ weeks gestation (by 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 per minute (bpm) but the ventricular rate was variable. There were frequent premature ventricular contractions and couplets, and short (3-4 beat) runs of tachycardia (Fig. 1A). During non-sustained 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, IgG to cytomegalovirus and *Toxoplasma gondii* were negative. The QTc intervals determined from 12-lead ECG recordings were in the normal range for the patient and fetus' father (424 ms and 383 ms, respectively). At 20 ½ weeks, a fetal magnetocardiogram (fMCG) revealed frequent short episodes of polymorphic ventricular tachycardia consistent with TdP and a QTc interval of 604 msec (Fig. 1B & 1C). During 2 hours of data recording, AV block was not observed. An ultrasound the same day showed interval accumulation of pleural fluid and ascites consistent with hydrops fetalis. Treatment of the fetal arrhythmia was discussed with the family, however, because of the dire clinical status, the parents elected not to pursue treatment.

Echocardiography at 22 weeks gestation revealed severe cardiac dysfunction, more frequent and more prolonged episodes of ventricular tachycardia (Fig. 1D), and worsening hydrops fetalis. Although tachycardia cycle length was similar to that observed 2 weeks prior (Fig. 1A), the velocity of the Doppler signals were 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. Based on 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 mutations were identified in ten other LQTS genes interrogated by the commercial genetic test (*KCNQ1*, *KCNH2*, *KCNE1*, *KCNE2*, *KCNJ2*, *CACNA1C*, *CAV3*, *SCN4B*, *AKAP9*, *SNTA1*). The fetal proband was also homozygous for a common nonsynonymous variant, R558 (rs1805124). However, analysis of parental DNA identified no evidence of germline mosaicism suggesting that *SCN5A*-L409P occurred on a *de novo* basis in the fetus.

Functional Consequences of *SCN5A*-L409P/R558

We determined the functional consequences of the mutation by *in vitro* electrophysiological recording of heterologously expressed recombinant human cardiac sodium channels (Na_v1.5). To recapitulate the genotype of the fetus, we engineered the L409P mutation along with the R558 variant (combination allele designated as L409P/R558). Expression of mutant channels in tsA201 cells generated voltage-dependent sodium currents that exhibited several differences compared to wildtype (WT) Na_v1.5 including a trend toward reduced peak current density (Fig. 2A & B), significant depolarized shifts in the voltage-dependence of steady-state inactivation and conductance-voltage relationships (Fig. 2C; Supplemental Table S1) and markedly accelerated recovery from inactivation (Fig. 2D). Additionally, as compared to WT channels, L409P/R558 exhibited a 7-fold greater level of persistent sodium current measured as a percentage of peak current ($1.4 \pm 0.2\%$, Fig. 3A; Table 1). Increased persistent current is the most frequently observed functional disturbance exhibited by *SCN5A* mutations associated with LQTS,¹⁷ and can also explain abnormal inward current evoked by a slow depolarizing voltage ramp (Fig. 3B & C). These findings indicate that L409P/R558 causes dysfunction of cardiac sodium channels consistent with cardiac arrhythmia predisposition. However, these findings do not provide an explanation for the severe intrauterine presentation of ventricular arrhythmia.

Developmental regulation of *SCN5A* exon 6 splicing

We hypothesized that the severity of LQTS presentation in this fetus was due to more severe functional consequences conferred by the mutant genotype in the background of a fetal-expressed alternatively spliced *SCN5A* transcript. We focused on the developmentally timed alternative splicing of exon 6 that generates two splice isoforms differing by seven amino acid residues within a voltage-sensing domain (D3/S3-S4) originally described in neuroblastoma cells.¹⁸ We determined the relative levels of *SCN5A* mRNA transcripts containing the canonical exon 6 or the alternative exon 6A in human heart samples representing various developmental stages including the fetal period (28-33 weeks gestation), infancy (2-6 months of age) and adulthood (> 18 years of age).

In fetal human heart, we observed ~1.5-fold greater levels of *SCN5A* mRNA transcript containing exon 6A, encoding what we designated as fetal Na_v1.5, as compared with transcripts containing the canonical exon 6, encoding the adult splice variant of Na_v1.5 (Fig. 4). In hearts from infants, the relative abundance of adult and fetal Na_v1.5 mRNA was approximately equal, while in adult heart there was a 7.5-fold higher level of adult Na_v1.5 mRNA as compared to the fetal splice variant. The fetal (exon 6A) to adult (exon 6) Na_v1.5 expression ratio in adult heart was significantly different from both infant and fetal heart ($p < 0.0001$) whereas differences between fetal and infant heart expression ratios were not

significant ($p=0.06$). We conclude that *SCN5A* exhibits a developmental switch in exon 6/6A alternative splicing in human heart during early postnatal life.

Functional properties of WT and mutant fetal $Na_v1.5$

To test whether the functional consequences of L409P/R558 are different in fetal $Na_v1.5$, we engineered a recombinant human fetal $Na_v1.5$ cDNA and compared its electrophysiological properties to adult $Na_v1.5$. We demonstrated that fetal $Na_v1.5$ exhibits a significantly more positive midpoint (+9 mV shift in $V_{1/2}$) in the conductance-voltage relationship as compared with the adult splice variant, but no significant differences in peak current density, voltage-dependence of steady-state inactivation curve or recovery from inactivation (Supplemental Figure S1; Table S1). These data demonstrate intrinsic differences in biophysical properties between human fetal and adult $Na_v1.5$ splice isoforms.

We investigated the consequences of the common *SCN5A* variant R558 on the functional properties of fetal $Na_v1.5$ and compared these to the effect of the variant on the adult splice isoform. Whereas, R558 has minimal functional impact on adult $Na_v1.5$ (Supplemental Table S1), the expression of this variant in fetal $Na_v1.5$ demonstrates substantial effects including lower whole cell current density and a large persistent sodium current (Fig 5; Table 1). These findings demonstrate a considerable functional defect for the non-mutant allele.

Finally, we determined the functional consequences of L409P/R558 in fetal $Na_v1.5$. Compared with biophysical properties of this mutation in the adult isoform, L409P/R558 in fetal $Na_v1.5$ exhibited greater depolarizing shifts in steady-state inactivation and in conductance-voltage relationships (Fig. 6 and Supplemental Table S1), and greater persistent current (Fig 6; Table 1). Superimposed, normalized current traces (Fig. 6D) and quantitative analysis also illustrate slower activation rise time and slower kinetics of inactivation for fetal $Na_v1.5$ -L409P/R558 as compared with WT fetal $Na_v1.5$, functional defects that were not as prominent in the adult splice isoform (Supplemental Fig S2). Collectively, these observations indicate that both *SCN5A* alleles carried by this fetus, one with R558 alone and the other with L409P/R558, cause much more profound sodium channel dysfunction in the background of fetal $Na_v1.5$ splice isoform providing a plausible explanation for severe presentation of intrauterine LQTS.

Discussion

We report, to our knowledge, the earliest confirmed diagnosis of symptomatic LQTS in a 19-week fetus. We also demonstrated a plausible mechanism for the early onset and unusual severity of the condition that involves interaction of the causative *SCN5A* mutation with the product of a developmentally regulated alternative splicing event in this gene. Our findings help explain how genetic cardiac arrhythmia susceptibility including LQTS can contribute to fetal mortality.^{6, 19-21}

Clinical and genetic features of fetal LQTS

The phenotype we described in a second trimester fetus associated with a previously undocumented *de novo* *SCN5A* mutation included ventricular ectopy and occasional periods of ventricular tachycardia, followed by a rapid progression to sustained episodes of TdP, impaired ventricular systolic function and severe hydrops fetalis. The diagnosis of LQTS *in utero* was facilitated by using fMCG and confirmed post-mortem by genetic testing. This unusually severe, early onset fetal arrhythmia was without other features typical of intrauterine LQTS presentations. The most common early manifestation of fetal LQTS is sinus bradycardia, which has been documented in some cases at less than 25 weeks

gestation, whereas other rhythm disturbances associated with fetal LQTS including second-degree AV block and TdP are most typical between 28 and 40 weeks gestation.²²⁻²⁴

A fetal diagnosis of ventricular tachycardia should prompt an evaluation for LQTS. In a review of recent literature regarding ventricular tachycardia in the fetus, 9 of 22 cases were confirmed to have LQTS.^{6, 9, 23, 25, 26} Moreover, of the LQTS cases, only 4 survived the neonatal period, suggesting that fetuses presenting with ventricular tachycardia, especially TdP, due to LQTS have a particularly poor prognosis. Fetal LQTS should also be considered in the differential diagnosis of complex fetal arrhythmia with fetal hydrops or unexplained fetal loss even in the absence of a family history. While the prevalence of fetal demise with LQTS is unknown, it is noteworthy that 30% of stillbirths are unexplained²⁷ and *de novo* LQTS gene mutations may account for a portion of these cases.

Carriers of certain *SCN5A* mutations, many of which are *de novo*, may present with earlier onset and more severe congenital arrhythmia syndromes.^{2, 4, 5, 10-14} Maternal mosaicism for *SCN5A* mutation has also been described in association with recurrent third-trimester fetal hydrops or stillbirth.⁸ Interestingly, *SCN5A* mutations are present in the preponderance of reported fetal and perinatal LQTS cases whereas only ~10% of LQTS in older children and young adults is explained by this genotype. The high rate of reported *de novo* mutations in the perinatal period may reflect low heritability due to a survival disadvantage conferred by severe phenotypes. Our report taken together with previous literature suggests a diagnosis of LQTS, with particular suspicion for *SCN5A* mutations, should be entertained in a fetus with ventricular arrhythmia even in the absence of a family history or parental genetic diagnosis of LQTS.

Alternative *SCN5A* splicing potentiates LQTS mutation severity

The novel *SCN5A* mutation we identified in this study encodes a dysfunctional cardiac sodium channel, but the degree of channel dysfunction determined in the context of the canonical adult $\text{Na}_v1.5$ splice product did not explain the unusual severity and early onset of fetal arrhythmia. We considered that using the adult $\text{Na}_v1.5$ splice isoform to evaluate the functional consequences of a mutation associated with an intrauterine arrhythmia might be misleading if another molecular form of the channel was predominant in fetal heart. *SCN5A* undergoes alternative mRNA splicing to generate multiple isoforms of the protein.²⁸

Although many described splicing events have uncertain physiological significance, at least one major alternative splicing event could have implications for understanding severe fetal LQTS. Specifically, a developmentally regulated *SCN5A* splicing event involving selection between two alternative forms of exon 6 generates $\text{Na}_v1.5$ isoforms that differ at several amino acid residues within a voltage-sensor domain (D1/S3-S4).^{18, 28} This alternative $\text{Na}_v1.5$ splice variant is strongly expressed in neonatal mouse heart but is down-regulated later in development.²⁹ Evidence for developmentally regulated expression in humans was not previously been demonstrated. Here we show prominent expression of alternatively spliced $\text{Na}_v1.5$ mRNA incorporating exon 6A in fetal and infant heart. Previous studies have referred to this product of alternative splicing as a 'neonatal' *SCN5A*, but given the high level of expression we observed in fetal human heart (Fig. 4), we suggest that fetal $\text{Na}_v1.5$ is a more appropriate designation.

Hence, we investigated the functional consequences of the mutation in the fetal $\text{Na}_v1.5$ splice isoform. These experiments indicated that the common variant (R558) and the compound mutant genotype (L409P/R558), the two alleles carried by the proband, each conferred much greater functional defects on fetal $\text{Na}_v1.5$ than on adult $\text{Na}_v1.5$. In particular, the proportion of persistent current was substantial for channels with L409P/R558 (11% of peak current) as well as with the R558 common variant alone (3.7% of peak current, Table 1), and was also abnormal for mutant adult $\text{Na}_v1.5$. Therefore, this fetus

expressed mostly dysfunctional *SCN5A* alleles at the time of the presenting arrhythmia syndrome. The profound defects observed for mutant fetal Na_v1.5 channels help explain both the malignant character of the arrhythmia syndrome and the early onset of this condition *in utero*. The dysfunction of fetal Na_v1.5-R558 suggests that carriers of this common allele may have greater risk for fetal or perinatal arrhythmias, but this seems incongruous with genotype frequencies observed in the general adult population (<http://www.ncbi.nlm.nih.gov/snp/?term=rs1805124>). We speculate that R558 alone is not sufficient to evoke arrhythmia risk in the fetus but may act as a modifier of other variants as previously demonstrated for other *SCN5A* mutations having later clinical presentations.³⁰⁻³³ We further speculate that impaired ventricular contractility was also the result of severe sodium channel dysfunction and related to either tachycardia as suggested by fetal Doppler studies (Fig. 1D) or disturbances in intracellular ionic homeostasis proposed for familial dilated cardiomyopathy associated with *SCN5A* variants.³⁴

Conclusion

In summary, we present a case of fetal LQTS identified early in mid-gestation due to a novel, *de novo* *SCN5A* mutation. Fetal LQTS may be an under-recognized cause of early fetal hydrops and unexplained fetal loss, and should be considered in the differential diagnosis of the fetus with rapidly progressive heart failure and complex arrhythmia even if family history is negative. The unusual severity and early onset of arrhythmia in this case were explained by profound dysfunction of the mutant sodium channel carried by the fetus that was revealed only by considering the correct molecular context, a fetal-expressed alternatively spliced *SCN5A* transcript. Our findings demonstrate an important contribution of developmentally regulated alternative *SCN5A* splicing to the genetic risk for prenatal life-threatening cardiac arrhythmia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding Sources: This work was supported by grants from the National Institutes of Health (HL083374 to A.L.G.; HL063174 to R.T.W.; and HL69712 to D. W. B.).

References

1. Schwartz, PJ.; Crotti, L. Long QT and short QT syndrome. In: Zipes, DP.; Jalife, J., editors. Cardiac Electrophysiology: From Cell to Bedside. Fifth. Philadelphia: Elsevier/Saunders; 2009. p. 731-744.
2. Wedekind H, Smits JP, Schulze-Bahr E, et al. De novo mutation in the SCN5A gene associated with early onset of sudden infant death. *Circulation*. 2001; 104:1158–1164. [PubMed: 11535573]
3. Schwartz PJ, Priori SG, Dumaine R, et al. A molecular link between the sudden infant death syndrome and the long-QT syndrome. *N Engl J Med*. 2000; 343:262–267. [PubMed: 10911008]
4. Schulze-Bahr E, Fenge H, Etzrodt D, et al. Long QT syndrome and life threatening arrhythmia in a newborn: molecular diagnosis and treatment response. *Heart*. 2004; 90:13–16. [PubMed: 14676229]
5. Wang DW, Crotti L, Shimizu W, et al. Malignant perinatal variant of long-QT syndrome caused by a profoundly dysfunctional cardiac sodium channel. *Circ Arrhythm Electrophysiol*. 2008; 1:370–378. [PubMed: 19808432]
6. Hofbeck M, Ulmer H, Beinder E, Sieber E, Singer H. Prenatal findings in patients with prolonged QT interval in the neonatal period. *Heart*. 1997; 77:198–204. [PubMed: 9093034]
7. Ohkuchi A, Shiraishi H, Minakami H, et al. Fetus with long QT syndrome manifested by tachyarrhythmia: a case report. *Prenat Diagn*. 1999; 19:990–992. [PubMed: 10521830]

8. Miller TE, Estrella E, Myerburg RJ, et al. Recurrent third-trimester fetal loss and maternal mosaicism for long-QT syndrome. *Circulation*. 2004; 109:3029–3034. [PubMed: 15184283]
9. Beinder E, Grancay T, Menendez T, Singer H, Hofbeck M. Fetal sinus bradycardia and the long QT syndrome. *Am J Obstet Gynecol*. 2001; 185:743–747. [PubMed: 11568808]
10. Bankston JR, Yue M, Chung W, et al. A novel and lethal de novo LQT-3 mutation in a newborn with distinct molecular pharmacology and therapeutic response. *PLoS ONE*. 2007; 2:e1258. [PubMed: 18060054]
11. Ten Harkel AD, Witsenburg M, de Jong PL, et al. Efficacy of an implantable cardioverter-defibrillator in a neonate with LQT3 associated arrhythmias. *Europace*. 2005; 7:77–84. [PubMed: 15670972]
12. Kehl HG, Haverkamp W, Rellensmann G, et al. Images in cardiovascular medicine. Life-threatening neonatal arrhythmia: successful treatment and confirmation of clinically suspected extreme long QT-syndrome-3. *Circulation*. 2004; 109:e205–e206. [PubMed: 15136511]
13. Yamagishi H, Furutani M, Kamisago M, et al. A de novo missense mutation (R1623Q) of the SCN5A gene in a Japanese girl with sporadic long QT syndrome. *Hum Mutat*. 1998; 11:481. [PubMed: 10200053]
14. Chang CC, Acharfi S, Wu MH, et al. A novel SCN5A mutation manifests as a malignant form of long QT syndrome with perinatal onset of tachycardia/bradycardia. *Cardiovasc Res*. 2004; 64:268–278. [PubMed: 15485686]
15. Wang DW, Desai RR, Crotti L, et al. Cardiac sodium channel dysfunction in sudden infant death syndrome. *Circulation*. 2007; 115:368–376. [PubMed: 17210841]
16. Gellens ME, George AL, Chen L, et al. Primary structure and functional expression of the human cardiac tetrodotoxin-insensitive voltage-dependent sodium channel. *Proc Natl Acad Sci USA*. 1992; 89:554–558. [PubMed: 1309946]
17. Bennett PB, Yazawa K, Makita N, George AL Jr. Molecular mechanism for an inherited cardiac arrhythmia. *Nature*. 1995; 376:683–685. [PubMed: 7651517]
18. Ou SW, Kameyama A, Hao LY, et al. Tetrodotoxin-resistant Na⁺ channels in human neuroblastoma cells are encoded by new variants of Nav1.5/SCN5A. *Eur J Neurosci*. 2005; 22:793–801. [PubMed: 16115203]
19. Ackerman MJ, Siu BL, Sturner WQ, et al. Postmortem molecular analysis of SCN5A defects in sudden infant death syndrome. *JAMA*. 2001; 286:2264–2269. [PubMed: 11710892]
20. Arnestad M, Crotti L, Rognum TO, et al. Prevalence of long-QT syndrome gene variants in sudden infant death syndrome. *Circulation*. 2007; 115:361–367. [PubMed: 17210839]
21. Berul CI. Neonatal long QT syndrome and sudden cardiac death. *Prog Pediatr Cardiol*. 2000; 11:47–54. [PubMed: 10822189]
22. Lin MT, Hsieh FJ, Shyu MK, et al. Postnatal outcome of fetal bradycardia without significant cardiac abnormalities. *Am Heart J*. 2004; 147:540–544. [PubMed: 14999207]
23. Tomek V, Skovranek J, Gebauer RA. Prenatal diagnosis and management of fetal Long QT syndrome. *Pediatr Cardiol*. 2009; 30:194–196. [PubMed: 18709401]
24. Horigome H, Nagashima M, Sumitomo N, et al. Clinical characteristics and genetic background of congenital long-QT syndrome diagnosed in fetal, neonatal, and infantile life: a nationwide questionnaire survey in Japan. *Circ Arrhythm Electrophysiol*. 2010; 3:10–17. [PubMed: 19996378]
25. Simpson JM, Maxwell D, Rosenthal E, Gill H. Fetal ventricular tachycardia secondary to long QT syndrome treated with maternal intravenous magnesium: case report and review of the literature. *Ultrasound Obstet Gynecol*. 2009; 34:475–480. [PubMed: 19731233]
26. Cuneo BF, Ovidia M, Strasburger JF, et al. Prenatal diagnosis and in utero treatment of torsades de pointes associated with congenital long QT syndrome. *Am J Cardiol*. 2003; 91:1395–1398. [PubMed: 12767447]
27. Vanderwielen B, Zaleski C, Cold C, McPherson E. Wisconsin stillbirth services program: A multifocal approach to stillbirth analysis. *Am J Med Genet A*. 2011; 155:1073–1080. [PubMed: 21480484]

28. Schroeter A, Walzik S, Blechschmidt S, et al. Structure and function of splice variants of the cardiac voltage-gated sodium channel Nav1.5. *J Mol Cell Cardiol.* 2010; 49:16–24. [PubMed: 20398673]
29. Chioni AM, Fraser SP, Pani F, et al. A novel polyclonal antibody specific for the Nav1.5 voltage-gated Na⁺ channel ‘neonatal’ splice form. *J Neurosci Methods.* 2005; 147:88–98. [PubMed: 16111763]
30. Ye B, Valdivia CR, Ackerman MJ, Makielski JC. A common human SCN5A polymorphism modifies expression of an arrhythmia causing mutation. *Physiol Genomics.* 2003; 12:187–193. [PubMed: 12454206]
31. Viswanathan PC, Benson DW, Balser JR. A common SCN5A polymorphism modulates the biophysical effects of an SCN5A mutation. *J Clin Invest.* 2003; 111:341–346. [PubMed: 12569159]
32. Poelzing S, Forleo C, Samodell M, et al. SCN5A polymorphism restores trafficking of a Brugada syndrome mutation on a separate gene. *Circulation.* 2006; 114:368–376. [PubMed: 16864729]
33. Shinlapawittayatorn K, Du XX, Liu H, et al. A common SCN5A polymorphism modulates the biophysical defects of SCN5A mutations. *Heart Rhythm.* 2011; 8:455–462. [PubMed: 21109022]
34. Nguyen TP, Wang DW, Rhodes TH, George AL Jr. Divergent biophysical defects caused by mutant sodium channels in dilated cardiomyopathy with arrhythmia. *Circ Res.* 2008; 102:364–371. [PubMed: 18048769]

Abbreviations

LQTS	long-QT syndrome
fMCG	fetal magnetocardiography
AV	atrioventricular
WT	wild type
TdP	torsade de pointes

Figure 1. Fetal doppler echocardiogram and magnetocardiogram

(A) Pulsed wave Doppler of the fetal aorta at 20 weeks gestation. Normal conducted beats (red arrows) are interrupted by frequent premature beats (PBs), and 2-3 beat runs of tachycardia with variable cycle length (white arrows). The flow velocity is maintained during the short runs of tachycardia, suggesting stroke volume is only slightly diminished. (B) Signal averaged 'butterfly plot' determined by fetal magnetocardiography during sinus rhythm at 20-6/7 weeks gestation illustrating T-wave alternans, a QTc interval of 604 msec, normal PR interval and a QRS duration that was slightly prolonged for age (0.69 msec). (C) Representative rhythm trace obtained by fetal magnetocardiography at 20-6/7 weeks gestation. Arrows indicate sinus beats interrupted by two episodes of non-sustained polymorphic ventricular (V) tachycardia with varying cycle length (400 to 200 msec). (D) Pulsed wave Doppler of the middle cerebral artery at 22 weeks gestation illustrating fetal arrhythmia. At this time, the fetus was severely hydropic with very poor systolic function (not shown). The tracing shows the onset of a sustained period (>3500 msec) of ventricular tachycardia (white arrows), initiating by a premature beat (PB). During the tachycardia, there is decreased stroke volume as evidenced by the extremely low velocity Doppler signals. The tachycardia cycle length was 480 to 210 ms.

Figure 2. Biophysical properties of WT and mutant sodium channels

(A) Representative whole-cell current recordings from cells expressing either WT (Adult $\text{Na}_V1.5\text{-H558}$) or mutant (Adult $\text{Na}_V1.5\text{-L409P/R558}$) channels (voltage protocol shown as inset). (B) Current-voltage relationships for WT ($n = 10$) and mutant ($n = 10$) channels. Current was normalized to cell capacitance to give a measure of current density. (C) Superimposed curves representing the voltage dependence of steady-state inactivation (left y-axis) and conductance-voltage (right y-axis) relationships for WT and mutant channels. Lines represent average fits of the data with Boltzmann functions. (D) Time course of recovery from inactivation recorded using the illustrated voltage protocol. Biophysical fit parameters for all experiments are provided in Supplemental Table S1.

Figure 3. *SCN5A*-L409P/R558 exhibits increased persistent current

(A) Representative tetrodotoxin (TTX)-sensitive currents were normalized to the peak current measured at -30mV during a 200 ms depolarization to illustrate persistent current. The inset represents the same data plotted on an expanded vertical scale. Summary data are provided in Table 1. (B) WT and mutant currents elicited by voltage ramps defined by the inset. (C) Amount of charge moved between -70 and -30mV normalized to peak current and quantified. Charge movement was significantly ($p < 0.02$) greater for mutant (10.2 ± 4.3 pC/nA, $n = 9$) than for WT (5.1 ± 4.0 pC/nA, $n = 9$) channels.

Figure 4. Developmental timing of SCN5A exon alternative splicing

Expression ratio (exon 6A / exon 6) of *SCN5A* mRNA transcripts expressed in human fetal (n = 4), infant (n = 4) and adult (n = 24) hearts determined by quantitative real-time RT-PCR. Differences among groups was significant at $p < 0.0001$ (one-way ANOVA with Tukey test).

Figure 5. Functional consequences of R558 variant on fetal-Na_v1.5

(A) Current density-voltage relationships recorded from cells expressing either WT (labeled Fetal-Na_v1.5-H558; n = 11) or variant (Fetal Na_v1.5-R558; n = 14) channels (voltage protocol same as in Fig. 2A). (B) Conductance-voltage relationships for WT and variant channels. (C) Steady-state voltage dependence of inactivation for WT (n = 10) and variant channels (n = 10). In (B) and (C) lines represent average fits of the data with Boltzmann functions. (D) Averaged TTX-sensitive persistent currents measured at -30 mV during a 200 ms depolarization and normalized to peak current (n = 7). The inset represents the same data plotted on an expanded vertical scale. Biophysical fit parameters for all experiments are provided in Supplemental Table S1, and magnitude of persistent current is provided in Table 1.

Figure 6. Expression in fetal-Na_v1.5 potentiates effect of SCN5A-L409P/R558

(A) Current density-voltage relationships comparing WT and mutant channels expressed in either adult or fetal Na_v1.5 channels (voltage protocol same as in Fig. 2A; $n = 10$ for all groups). (B) Conductance-voltage relationships for WT and mutant channels. (C) Steady-state voltage dependence of inactivation for WT and mutant channels ($n = 10-12$). In (B) and (C) lines represent average fits of the data with Boltzmann functions. (D) Representative TTX-sensitive persistent currents measured at -30 mV during a 200 ms depolarization and normalized to peak current. Biophysical fit parameters for all experiments are provided in Supplemental Table S1, and magnitude of persistent current is provided in Table 1.

Table 1
Persistent TTX-sensitive sodium current measured at -30 mV

	Persistent Current (% of peak I_{Na})	Current Density (pA/pF)	n
Na _v 1.5-H558	0.2 ± 0.4	0.4 ± 0.1	8
Na _v 1.5-R558	0.4 ± 0.2	0.4 ± 0.2	3
Na _v 1.5- L409P/R558	$1.4 \pm 0.2^{**}$	2.5 ± 0.9	7
Fetal Na _v 1.5-H558	$0.7 \pm 0.3^*$	1.3 ± 0.9	4
Fetal Na _v 1.5-R558	$3.7 \pm 0.9^{**\dagger}$	$3.5 \pm 1.2^*$	7
Fetal Na _v 1.5-L409P/R558	$11.1 \pm 1.5^{**\dagger}$	$4.4 \pm 1.2^*$	10

* $P < 0.05$ compared to Na_v1.5-H558;

** $P < 0.005$ compared to Na_v1.5-H558;

† $P < 0.05$ compared to Fetal Na_v1.5 -H558;

† $P < 0.005$ compared to Fetal Na_v1.5-H558

Statistical comparisons made using ANOVA with Dunn's post hoc test