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# Enhanced impact of *SCN5A* mutation associated with long QT syndrome in fetal splice isoform

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Congenital long QT syndrome (LQTS) is an inherited syndrome characterized by prolongation of the QT interval on the electrocardiogram and an increased susceptibility to life-threatening ventricular arrhythmias. Mutations in the *SCN5A* gene, which encodes the α-subunit of the cardiac $Na^+$ channel, represent the third most common cause of LQTS, behind mutations in potassium channel genes *KCNQ1 and KCNH2*. Moreover, mutations in *SCN5A* have been linked to other types of inherited channelopathies, including the Brugada syndrome (BRS1), progressive familial heart block type 1 (PFHBI), sick sinus syndrome type 1 (SSS1), idiopathic ventricular fibrillation (IVF), familiar atrial standstill, dilated cardiomyopathy type 1E (CMD1E), and sudden infant death syndrome (SIDS)[1]. In total, more than 400 unique DNA variants have been reported in *SCN5A*, of which at least more than 80 mutations were linked to LQTS alone (see inherited arrhythmia data base: http://www.fsm.it/cardmoc/).

Mutations in the *SCN5A* gene associated with LQTS typically cause a gain-of-function phenotype resulting in enhanced $Na^+$ entry into the cardiomyocyte during the repolarization period [2]. Each $Na^+$ channel α-subunit (Nav1.5) consists of four structurally homologous domains (DI-DIV), each comprising six transmembrane segments (S1-S6). Most mutations in Nav1.5 disrupt fast inactivation and thereby cause a persistent (or sustained) $Na^+$ current. However, some $Na^+$ channel mutations rather enhance window currents when inactivation occurs at more depolarized potentials, resulting in delayed repolarization in the absence of persistent $Na^+$ current [3]. Other biophysical mechanisms of Nav1.5 dysfunction causally linked to LQTS include faster recovery from inactivation, slower inactivation, and a larger peak $Na^+$ current ($I_{Na}$) density [1]. Regardless of the underlying mechanism, gain-of-function defects in Nav1.5 disrupt the delicate balance between depolarization and repolarization during the action potential plateau phase, thus delaying repolarization and increasing the risk of lethal ventricular arrhythmias.



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Postmortem studies have revealed that *SCN5A* mutations may be the most prevalent genetic cause of sudden infant death syndrome (SIDS), which is the unexpected, sudden death of a child under age 1 in which autopsy does not reveal an explainable cause of death [4]. Most *SCN5A* mutations found in SIDS victims cause biophysical phenotypes similar to those associated with mutations found in older children or adults with LQTS. However, a few SIDS-linked mutations in *SCN5A* exhibit sustained $I_{Na}$ only under acidic conditions, suggesting that environmental factors such as hypoxia or acidosis might contribute to the lethal arrhythmias in susceptible infants [5].

In addition, several papers have reported even earlier, prenatal diagnosis of LQTS linked to *SCN5A* mutations. Such variants were identified in several parts of the channel (e.g., R43Q, L619F, F627L, A1186T, P1332L, F1473C, F1486del, R1623Q, V1763M, N1774D) [6–9]. The most common prenatal manifestations of LQTS include sinus bradycardia and atrioventricular block, presumably due to excessive refractory periods related to delayed repolarization. In addition, irregular heart rates due to ventricular ectopy and ventricular tachycardia are commonly observed. In more than half of all published cases, *in utero* demise occurred during the third trimester [6–9]. Previous biophysical analysis of the abovementioned *SCN5A* variants did not reveal biophysical defects distinct from those described for *SCN5A* mutations found in individuals with a postnatal diagnosis of LQTS. Therefore, it has remained unclear why fetuses with *SCN5A* mutations exhibit more severe repolarization defects and higher mortality rates compared to older mutation carriers.

In the current issue of Heart*Rhythm*, Murphy *et al*. [10] described an interesting case report of a fetus carried by a 29-year-old primiparous, otherwise healthy woman, who was diagnosed at 20 weeks of gestation with frequent premature ventricular contractions, which represents the earliest described case of fetal LQTS. The fetus developed episodes of ventricular ectopy, which soon thereafter progressed into polymorphic ventricular tachycardia, extreme QTc interval prolongation, and hydrops fetalis. Because of the extent of the clinical deterioration, pregnancy was terminated at the request of the family. Genetic analysis revealed a novel, *de novo*, heterozygous missense mutation (L409P) in *SCN5A*, as well as homozygosity for the common nonsynonymous variant R558 [11].

The biophysical features of the mutant $Na^+$ channels were studied using whole cell patch clamp of tsA201 cells expressing recombinant Nav1.5 channels with mutation L409P and polymorphism R558. These Nav1.5-L409P/R558 mutant channels exhibited reduced peak current density, depolarized shifts in voltage-dependence of activation and inactivation, and faster recovery from inactivation. In addition, a much larger persistent $Na^+$ current was measured, which is a common feature among most LQTS-linked $Na^+$ channel mutants [1].

Next, the authors explored the interesting hypothesis that the severe clinical manifestations of LQTS in the affected fetus were due to alternative splicing of a *SCN5A* transcript expressed during the fetal period. In human fetal hearts, alternative exon 6A is more abundant than in infant or adult heart. Compared to the adult isoform, fetal Nav1.5-L409P/R558 channels exhibited a more pronounced shift in fast inactivation and an even larger persistent $Na^+$ current. Moreover, the fetal isoform exhibited a slower activation rise time and slower inactivation kinetics, similar to previous reports [12]. These exacerbated changes in

$Na^+$ channel gating may explain the severity of the clinical phenotype in the fetus with the L409P mutation and R588 polymorphism.

The replacement of exon 6 by exon 6a as a result of alternative splicing results in the substitution of 7 amino acids in the fetal Nav1.5 channel. Onkal *et al.* [12] demonstrated that replacement of a single negatively charged aspartate at position 211 in the adult isoform with a positively charged lysine residue in the fetal isoform introduces a positive charge in the S3 domain adjacent to the S4 voltage sensor of domain I. This particular amino acid substitution was shown to be primarily responsible for the functional effects of exon 6 splicing on Nav1.5 channel parameters.

The present study by Murphy *et al.* [10] revealed that the electrophysiological effects of the R558 polymorphism were similar in the adult and fetal Nav1.5 isoforms. However, when the L409P mutation was added to the R558 polymorphism, more pronounced $Na^+$ channel dysfunction was observed in case of the fetal splice variant. This suggests that alternative splicing of the fetal isoform might be the primary reason for the severe fetal manifestation of arrhythmias in carriers of *SCN5A* mutations. Since most genes causally linked to LQTS are also subject to alternative splicing, it would be interesting to determine whether the effects of mutations in other cardiac ion channels are also more potent in the fetal splice variants.

Finally, it was shown that the R558 polymorphism independently contributed to enhancement of Nav1.5 channel dysfunction caused by the L409P mutation. This observation highlights the importance of *SCN5A* polymorphisms in terms of $Na^+$ channel electrophysiology. For example, polymorphism S1103Y, which is commonly found in African Americans, has been linked to SIDS [13]. Another variant, R1193Q, commonly found in Asians [14] may also increase the risk of SIDS and prenatal death [15]. Moreover, polymorphism V1951L found in Latinos [16] also modulates the biophysical effects of *SCN5A* mutations [17], and has been identified in a victim of SIDS [5].

In conclusion, the paper by Murphy *et al.* [10] suggests that the unusual severity and early onset of ventricular arrhythmias in a fetus with an *SCN5A* mutation could be attributed to synergistic effects of a disease-causing mutation, a polymorphism, and an alternative splice variant. It would be important to consider the contributions of each of these three factors in future studies of *SCN5A* variants associated with fetal or perinatal arrhythmias and sudden cardiac death.

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