The Role of the Cardiac Sodium Channel in Perinatal-Early Infant Mortality

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Abstract

The cardiac sodium channel gene, *SCN5A*, plays an important role in arrhythmias of genetic origin. With exceptions, loss-of-function mutations become more important in adult life whereas gain-of-function mutations can manifest their clinical impact very early, also in the perinatal period. The best known disease caused by the latter variants is the long QT syndrome type 3 (LQT3), which tends to manifest in adolescent age. However, when symptoms appear in the first year of life the likelihood of cardiac arrest and sudden death is very high. *SCN5A* mutations can also produce life-threatening events much earlier, in the first few months of life (causing Sudden Infant Death Syndrome, SIDS), and even before birth (causing Intrauterine Unexplained Fetal Death, IUFD). Functional genetic variants in LQTS genes are present in 10-15% of SIDS and 7-8% of IUFDs, and their majority is represented by mutations affecting INa. Cases with severe LQT3 manifesting in the perinatal early-infant period, are associated with very marked QT prolongations which make them easily identifiable by ECG screening in the first month of life thus enhancing the chances of effective preventive therapies. There are differences between the *SCN5A* mutations producing LQT3 or SIDS/IUFD, and they are related to topology. Furthermore, comparisons of the genetic variants in LQT3, SIDS/IUFD, and general population allow to understand both the truncated curve of the population with the most severe LQT3 mutations (usually “de novo”) and the milder favoring role of some of the variants found in SIDS/IUFD which probably require other factors to trigger sudden death.
Keywords: sodium channel, SIDS, stillbirth, IUFD, long QT syndrome, perinatal mortality, genetics

Key Points:

1) The cardiac sodium channel plays an important role in arrhythmias of genetic origin and it is implicated in perinatal mortality.

2) Channelopathies have been implicated in 15% of SIDS cases and genetic variants with a functional effect on SCN5A or in sodium channel ancillary proteins are present in the vast majority of them.

3) Genetic variants with a functional effect in LQTS genes are present in 8.8% of IUFD cases and SCN5A plays a major role.

4) Cases with severe LQT3 manifesting in the perinatal early-infant period, are associated with very marked QT prolongations which make them easily identifiable by ECG screening in the first month of life, thus enhancing the chances of effective preventive therapies.

5) There are differences between SCN5A mutations producing severe early-onset LQT3 and SIDS/IUFD in terms of topology and frequency in the general population. This observation translates in pathophysiological considerations.
Introduction

Mortality in the perinatal and infant period is an important public health issue and indeed, their rate of occurrence represents a commonly used indicator of the health status of any given population (1). A wide range of causes are associated with such a tragic event (i.e. a variety of diseases including infections and congenital defects, poor nutritional status, etc.) and their relative importance varies significantly in different countries, according to the socio-economic conditions (2). The identification of the causes is essential to allow the implementation of focused preventive approaches (2). However, still a significant number of these deaths (25-40% of stillbirths and approximately 10% of neonatal demises) are unexplained after a thorough post-mortem examination (3-4). It is among these that sodium channel dysfunctions play a role.

Ion channel diseases, the so called “channelopathies”, are a group of genetically transmitted heart diseases, characterized by a morphological normal heart and by a predisposition to life-threatening arrhythmias which could cause sudden cardiac death (SCD) even very early in life (5). Whenever SCD is the first clinical manifestation of the disease, these cases would be labelled according to the age of occurrence, as sudden unexplained deaths (SUD) (5,6), sudden infant death syndrome (SIDS) (7,8) or intrauterine unexplained fetal deaths (IUFD) (3). Channelopathies have been recognized as the cause of the SCD in approximately 35% of SUD, 15% of SIDS and 9% of IUFD (3,6-8) and mutations in SCN5A have been identified in all subgroups.

The cardiac sodium channel

The cardiac sodium channel, a member of the voltage dependent family of ion channels, is a transmembrane protein involved in the generation and transmission of action potential. It is a large molecular complex containing α-subunit, four ancillary β-subunits, and several regulatory proteins (9). The α-subunit of the channel (designated Na\textsubscript{v}1.5) forming the ion conducting pore is encoded
by the SCN5A gene (UCSC uc021wvo.1; OMIM #603830) located on the short arm of chromosome 3 (3p21-24) (10). Na1.5 consists of four homologous domains, from DI to DIV, with three interdomain linkers. These linkers, as well as the N- and C-terminus of the protein, are located inside the cytoplasm of the cell.

The Na\(^+\) channel isoform Na,1.5 is the predominant \(\alpha\) subunit in the human heart (11); however, other four splice variants are recognized, known respectively as Na,1.5a (exon 18 deletion), Na,1.5c (glutamine 1077 insertion), Na,1.5d (deletion in exon 17) and Na,1.5e (alternative neonatal exon 6a) (12). Specifically, exon 6, encoding part of the S3/S4 region in DI, is known to be alternatively spliced through developmental stages, being different in neonatal and adult heart (13). The neonatal (Na,1.5e) and the adult isoform (Na,1.5) behave differently in patch-clamp experiments (14) and this should be considered when studying sodium channel mutations implicated in perinatal mortality.

Sodium channel mutations causing an increase in persistent inward Na\(^+\) current during myocardial repolarization are referred to as gain-of-function mutations and are responsible for the type 3 variant of Long QT Syndrome (LQTS), aka LQT3 (15). In this subgroup of LQTS patients most cardiac events occurs while patients are at rest or asleep (16) (Figure 1), consistent with what observed in SIDS cases. Loss-of-function mutations in the sodium channel gene, can result in different channelopathies, the main one being the Brugada Syndrome (BrS) (5). Currently there are more than 300 known SCN5A mutations associated with the Brugada phenotype; while among the minor genes implicated in the disease (17), around half are proteins modulating the sodium channel function. As the clinical manifestations of BrS usually occur in adulthood, loss-of-function mutations in the sodium channel gene are usually less implicated in perinatal mortality compared to gain-of-function mutations.
Role of SCN5A in neonatal sudden unexpected deaths

Sudden infant death syndrome (SIDS) remains the leading cause of mortality in the first year of life and its actual prevalence is around 0.5-0.6 per 1,000 live births (7). During the Second International Conference on Causes of Sudden Death in Infants, held in Seattle in 1970 (18), Beckwith provided its first classic definition: *SIDS is the sudden death of any infant or young child which is unexpected by history, and in which a thorough postmortem examination fails to demonstrate an adequate cause of death.* This definition remains valid, despite several revisions by the National Institute of Child Health and Human Development (NICHD), with the specification that the victim had to be below age 1-year, that the death had to occur during sleep and including not only a complete postmortem examination, but also an evaluation of clinical history and a review of the death scene (19).

Many different theories have been developed during the years to explain these deaths *sine materia* and many different risk factors have been identified, some maternal (i.e. multiple pregnancies, young age, smoking, drug intake, alcohol use), some infant-specific (i.e. prone sleeping, prematurity, low birth weight) and some environmental (i.e. bed-sharing with parents or siblings, winter months, low socioeconomic status, lack of breastfeeding) (7). Progressively it has been accepted that SIDS is a multifactorial disease, i.e. many different causes can provoke the SCD of an infant and multiple factors, by themselves insufficient, may act synergistically to induce sudden death. The latter concept represents the so called *triple risk model* (20), that hypothesizes that SIDS may occur only if a vulnerable infant is exposed to one or more exogenous stressors, during a critical developmental period (20). Not in contrast with the *triple risk model* is the *cardiac hypothesis*, formally advanced in 1976 (21). According to this hypothesis, some cases of SIDS may be due to a lethal cardiac arrhythmia, i.e. ventricular fibrillation, through a mechanism similar to the one acting in the congenital long QT syndrome (LQTS). In this context, a mutation in a cardiac ion
channel gene may render an infant vulnerable, and whenever a trigger is present, during a critical developmental period, SIDS may occur (7).

The proof of concept that LQTS can cause SIDS, came in 2000 (22), when a 44-day-old infant was found by the parents cyanotic, apneic and pulseless. If this infant would not have been found by the parents in time to be rushed to the nearby hospital and resuscitated from documented ventricular fibrillation, it would have been a typical SIDS case. The infant had a corrected QT interval of 648 ms and a \textit{de novo} mutation (S941N) in \textit{SCN5A} was identified (22) (Figure 2). In the following years, the relevance of LQTS was evaluated in different cohorts of SIDS cases (23-26) and the presence of functional mutations in the LQTS genes was identified in 10-15% of the cases. The Na\textsuperscript{+} channel in particular plays a major role in SIDS. Indeed, more than half of the mutations identified in population-based cohort studies are related to the Na\textsuperscript{+} channel (23-27). Specifically, in addition to mutations in the \(\alpha\) subunit of the Na\textsuperscript{+} channel (encoded by the \textit{SCN5A} gene), mutations in the \(\beta\) subunit-encoding genes (\textit{SCN1Bb, SCN3B, SCN4B}) and in Na\textsubscript{v},1.5 regulatory genes (\textit{CAV3, SNTA1, GPD1L}) were identified (Figure 3). The relevance of Na\textsuperscript{+} channel dysfunctions in the genesis of SIDS is also in line with the clinical evidence that both in LQT3 and in SIDS the life-threatening events occur at rest or during sleep (7,16).

As previously mentioned, an impaired cardiac Na\textsuperscript{+} channel can lead both to LQTS (gain-of-function) and BrS (loss-of-function), depending on the functional effect of the specific mutation. In the literature there are also some examples of SIDS in which the electrophysiological properties of the \textit{SCN5A} mutations are associated with a more Brugada-like phenotype (26).

Table 1 provides the list of \textit{SCN5A} mutations and mutations in Na\textsubscript{v},1.5 related-genes (23,26,28-35) identified in SIDS cases.

Recently, Andreasen et al (36) questioned the role of \textit{SCN5A} variants identified in SIDS cases, as they observed their presence also in the general population, as reported in the Exome Variant Server
(EVS) database (37). In our view, this could simply mean that variants present in the EVS are not sufficient to cause SCD by themselves, but they could act as favouring factors, according to the triple risk model (20).

**Role of SCN5A in IUFD**

Intrauterine unexplained fetal death (IUFD), including miscarriages (fetal losses <20\textsuperscript{th} week of gestation) and stillbirths (fetal losses ≥20\textsuperscript{th} week), is a major public health problem with significant impact especially on the mothers. Stillbirth has an incidence of 6.05 per 1,000 live births (38). In 70% of cases a probable or a possible cause to explain the demise can be identified (40% placental, 21.5% fetal, 12.7% maternal causes), whereas the remaining cases are unexplained (39,40). Cardiac channelopathies have been shown to contribute to sudden death in children and infants with an inconclusive autopsy (7,8,24). Therefore, it was logical to think that the same mechanism causing sudden cardiac death in the first few months of life, could cause SCD also just before birth (41). The first proof of this concept came from a study by Hoorntje et al, which found a homozygous premature truncation of the \textit{KCNH2} protein in a stillbirth case and in the sister who was born premature in distress, due to ventricular arrhythmia in the presence of severe QT prolongation (42). Later on, the \textit{SCN5A}-R1623Q mutation was identified as the cause of two stillbirths and of a malignant perinatal LQTS, requiring cardiac transplantation, in the three siblings (43); the mother had a mosaicism and this explains the possibility of transmission of such a severe mutation (43).

The only study so far designed to evaluate the prevalence of LQTS mutations in a population of IUFD cases was published last year and included 91 cases (average gestational age at fetal death 26.3, range 14-41 weeks) (3). Through the analysis of the 3 main LQTS-genes (\textit{KCNQ1, KCNH2, SCN5A}), variants leading to \textit{in vitro} dysfunctional ion channels were identified in 8 IUFD cases (8.8%). Three cases (3.3%) were carrying mutations, never identified in controls and associated with a functional effect; specifically, two mutations (\textit{KCNQ1}-A283T, -R397W), were associated with a marked reduction of the \textit{I_{Ks}} current, consistent with LQT1, whereas the HERG1b-R25W
mutation exhibited a loss-of-function consistent with in utero LQT2 (3). Five cases (5.5%) were carrying 3 rare genetic variants on SCN5A (T220I, R1193Q, P2006A), present in the general population with a very low frequency and demonstrated to be functionally relevant (3). As observed in SIDS, most of the genetic variants with a functional effect identified are located on SCN5A (Figure 4); and even if these variants are also present in the general population (36,37), they are significantly more prevalent in IUFD cases (p=0.0001), again suggesting their role as predisposing factors.

**Role of SCN5A in documented life-threatening arrhythmias in the perinatal period**

Long QT Syndrome patients with life-threatening arrhythmias in the first year of life represent a small subset of Romano-Ward patients (in LQTS International Registry 2% of 3,323 subjects) (47); however, their risk for subsequent cardiac arrest/sudden cardiac death in the following 10 years is particularly high (HR 23.4, p<0.01) and their response to beta-blocker therapy is poor (47,48).

This subgroup of patients, independently on the genotype, should be regarded as a subgroup of LQTS patients in whom traditional treatments are usually not effective and in whom an aggressive strategy is usually needed, at variance with the vast majority of the LQTS patients (5,15). Their ventricular arrhythmias usually start very early, sometimes also in the fetal period. Cuneo et al, studied 43 subjects exhibiting foetal arrhythmias potentially linked to LQTS (Torsades de Pointes, second degree AV block and sinus bradycardia) and evaluated the correlation with the LQTS genotype (49). Disease-causing variants in known LQTS-genes were found in 95% (38/40) of tested cases, 35 of them carrying a mutation in one of the major LQTS genes (23 KCNQ1, 6 KCNH2 and 6 SCN5A). Excluding the fetuses with only second degree AV block and sinus bradycardia, and focusing on the infants with life-threatening arrhythmias, who in the study were 7, it is interesting to observe that 5/7 (71%) were carrying a SCN5A mutation (49). Another interesting observation from this study was the overrepresentation of the SCN5A-R1623Q mutation, present in 4 of the 5 LQT3 cases with life-threatening arrhythmias (49) and already identified in association with very
severe and early-onset phenotypes (43,50). In the literature other very severe cases associated with de novo mutation in SCN5A have been reported (51-55), all presenting very prolonged QT and sign of electrical instability, such as 2:1 AV block and T-wave alternans.

Horigome et al (56), using a questionnaire collected 58 cases of LQTS from 33 institutions in Japan, in which the diagnosis has been done in fetal, neonatal or infant life (up to 1 year). Among these, 41 underwent genetic testing and 19 had life-threatening arrhythmias. In this cohort, at variance with what mainly present in literature, a greater prevalence of KCNH2 mutations was observed, while SCN5A mutations were identified in 26% of the cases.

In our own internal data-base, among 11 severe early onset LQTS cases, 5 have a mutation on SCN5A (48 and unpublished) (45%), 3 on calmodulin genes (57) (27%) and the remaining 3 are distributed equally among KCNH2, KCNQ1 and unknown genotype. Calmodulin mutations associated with these severe forms of LQTS cause impaired calcium affinity (57); however, how this produces severe cases long QT syndrome and arrhythmias is still under investigation given the multiple ion channels, including the sodium channel (58) targeted by calmodulin. Table 2 summarises all the SCN5A mutations so far identified in association with malignant early onset LQT3.

In summary, these data show the prominent role of SCN5A in inducing life-threatening conditions in the perinatal period (Figure 4). Given the observation that in adult life SCN5A mutations are present only in a minority of genotype-positive LQTS patients, it is reasonable to postulate a negative selection for SCN5A carriers in the perinatal and early-infant period.
Differences among the SCN5A mutations identified in SIDS, IUFD and LQT3 patients with life-threatening arrhythmias in the perinatal period.

In SIDS, IUFD and in neonatal LQT3, the majority of the genetic variants identified with a functional effect are located on SCN5A, and probably these variants are responsible for an adverse selection, as the picture is completely reversed in adult life (Figure 4). However, the genetic variants identified in SIDS, IUFD and neonatal LQT3, have a different topological distribution. Indeed, as shown in Figure 5, mutations located in the transmembrane and linker regions (the regions where variants present in controls are less frequently observed) (59) are significantly more present in neonatal LQT3 cases than in SIDS/IUFD (86% vs 22%, p=0.002). While the variants located in the N-terminal and in the interdomain linkers (the regions where variants present in controls are more frequently observed) (59), are 9.5% in neonatal LQT3 vs 44.4% in SIDS/IUFD, p=0.01. Furthermore, while none of the mutations identified in the cases with severe neonatal LQT3 are present in the EVS database, 47% of functional variants associated with SIDS/IUFD are also observed in the general population. These considerations support the concept that neonatal severe LQT3 cases are caused by mutations able per se to cause such a severe phenotype and are therefore not compatible with survival to adulthood. Indeed, the mutations identified in this subgroup of patients are mainly de novo. By contrast, the genetic variants identified in SIDS and IUFD cases have a wider range of clinical severity and some of them, identified also in the general population, are probably not able per se to cause SCD, while they can act as favouring factors. In further support of this view, we have observed that the SCN5A rare variants with a functional effect identified in SIDS and IUFD, also present in the EVS database, are significantly more prevalent in the SIDS/IUFD cohorts than in the general population (2.8% vs 1.5%, p=0.01). This observation is strengthened by the removal of SCN5A-V1951L and of SCN5A-F2004L, the most prevalent variants in EVS which were initially considered as potentially detrimental variants in the SIDS cohort (23), but not in the more recent IUFD cohort (3). Indeed, while the prevalence of these
two variants is similar in the SIDS cohort and in EVS, thus not supporting their role in increasing the risk of SCD, all the others are much more prevalent in SIDS and IUFD compared to the general population (12/634, 1.9% vs 45/6261, 0.7%, p=0.0019).

Clinical Implications

The concepts discussed above contribute to a better understanding of the mechanisms underlying a number of sudden deaths occurring shortly before or shortly after birth. Importantly, they also carry practical clinical implications.

It is rather evident that the cardiac sodium channel mutations associated with the highest risk and with the more malignant clinical course are those accompanied by very marked QT interval prolongations. As such, they could be easily identified even before the onset of the first episode of life-threatening arrhythmias, provided that an ECG is performed in the first weeks after birth. The European Society of Cardiology has published (60) guidelines for the interpretation of neonatal ECG and has clearly supported an ECG screening program to be performed in the first 2-3 weeks of life with the main objective to identify early on infants affected by LQTS, thus allowing early initiation of protective therapeutic strategies. A prospective ECG study in over 40,000 2-3-week-old infants has demonstrated that neonatal ECG screening can identify the affected infants with subsequent genetic confirmation (61). The cost-benefit ratio in Europe is very favorable (62) and it is difficult to justify the still ongoing resistance to what would be a simple and very effective screening program which, by the early use of appropriate therapeutic strategies, could lead to a significant reduction of avoidable tragedies (63).-
Acknowledgments: We are grateful to Pinuccia De Tomasi for expert editorial support, to Elisa Mastantuono for helping with Medline searches and to Carla Spazzolini for statistical support.
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TABLE 1: SIDS-associated genetic variants in the cardiac sodium channel gene and in its ancillary proteins.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Cardiac Functional Role</th>
<th>Genetic Variant</th>
<th>Reported Study [ref]</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCN5A</td>
<td>Nav1.5</td>
<td>α subunit of I&lt;sub&gt;Na&lt;/sub&gt; channel</td>
<td>S216L, delAL586-587, R680H, R1193Q (2 cases), T1304M, F1486L, V1951L, F2004L (3 cases), P2006A (2 cases)</td>
<td>23</td>
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<td></td>
<td></td>
<td></td>
<td>F532C, G1084S, F1705S</td>
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<td></td>
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<td></td>
<td>A997S, R1826H</td>
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<td>S524Y (2 cases), R689H, E1107K</td>
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<td>Q692K, R975W, S1333Y</td>
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<td>C72W, T78M</td>
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<td>SCN3B</td>
<td>Navβ3</td>
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<td>V36M, V54G</td>
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<tr>
<td>GPD1-L</td>
<td>G3PD1L</td>
<td>Glycerol-3-phosphate dehydrogenase 1-like</td>
<td>I124V, R273C</td>
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Note: Functionally significant variants are in bold; homozygous and double/triple mutation carriers are excluded.
TABLE 2: Genetic variants in the cardiac sodium channel gene found respectively in LQT3 infants with early malignant arrhythmias in utero or within the first year of life and in intrauterine fetal demises.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Cardiac Functional Role</th>
<th>Genetic Variant</th>
<th>Reported Study [ref]</th>
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<td>SCN5A</td>
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<td>G1631D (2 cases)</td>
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<td></td>
<td></td>
<td></td>
<td>R1623Q (7 cases)</td>
<td>43,49,50,our group unpublished</td>
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<td>V1763M (2 cases)</td>
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<td>T220I, S524Y, D772N,</td>
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<td>R1116Q, R1193Q (2 cases),</td>
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<td></td>
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<td>P2006A (2 cases)</td>
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</tbody>
</table>

Note: Functionally significant variants are in bold; homozygous and double/triple mutation carriers are excluded.
LEGENDS FOR FIGURES

**Figure 1:** Triggers for lethal and non-lethal cardiac events in the three main LQTS genotypes *(Modified from ref. 16)*

**Figure 2:** Electrocardiograms (ECGs) at the time of admission to the hospital (Panel A) and after the restoration of sinus rhythm by countershock (Panel B). At hospital admission, this 44-day-old infant had ventricular fibrillation (Panel A). After return to sinus rhythm, the ECG showed a QT interval extremely prolonged (QTc 648 ms) (Panel B), and also a clear T wave alternans. He was found to carry *SCN5A*-S941N, a *de novo* mutation. Whole-cell current traces measured in *Xenopus laevis* oocytes and recorded with a voltage-clamp protocol (Panel C). Representative wild-type and mutant (*SCN5A*-S941N) late Na\(^+\) currents, expressed as the percentage of the peak current that was tetrodotoxin-sensitive and recorded at 0 mV, were shown on the left. The relative amplitudes of the wild-type and mutant late sodium currents measured at 300 ms are showed on the right at different membrane potentials (P<0.001). Values are means (+SD) of seven experiments *(Modified from ref. 22)*.

**Figure 3:** Percentage of SIDS cases with functional genetic variants identified in cardiac channelopathy-genes and reported in population-based cohort studies. Comparison among SIDS carriers of mutations in channelopathy-associated genes, SIDS cases with functional variants affecting I\(_{Na}\), SIDS cases with functional variants located in *SCN5A* or in ancillary proteins forming the *SCN5A* macromolecular complex. The percentages are obtained according to Table 1.

**Figure 4:** Distribution of mutation carriers in the main two rectifier potassium channel genes (*KCNQ1, KCNH2*; in light blue) and in sodium channel (*SCN5A*; in red), for four different populations: adult patients, SIDS cases, intrauterine fetal demises and LQTS infants with life-
threatening arrhythmias in utero or within the first year of life. The distribution of variants between the two channels is similar in SIDS, IUFD and LQTS infants populations, all significantly different from the adults (p<0.0005). The percentages of functionally-relevant variants were calculated according to ref. 44 for LQTS adult subjects, to table 1 for SIDS cases, to table 2 for IUFD cases, to table 2 and ref. 45,46 for LQTS infants.

**Figure 5:** Distribution and location of functional variants reported in SIDS (light blue circles), IUFD (red circles) and LQT3 infants with life-threatening arrhythmias in utero or within the first year of life (yellow circles), in structural-functional domains of Na\textsubscript{v}1.5 (*Modified from ref. 9*).