Inherited arrhythmia syndromes

From genotype to phenotype in hiPSC-derived cardiomyocytes

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Chapter 1

The cardiac sodium channel gene *SCN5A* and its gene product $Na_v1.5$: role in physiology and pathophysiology

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ABSTRACT

The gene *SCN5A* encodes the main cardiac sodium channel $Na_V1.5$. This channel predominates the cardiac sodium current, I_{Na}, which underlies the fast upstroke of the cardiac action potential. As such, it plays a crucial role in cardiac electrophysiology. Over the last 60 years a tremendous amount of knowledge regarding its function at the electrophysiological and molecular level has been acquired. Furthermore, genetic studies have shown that mutations in *SCN5A* are associated with multiple cardiac diseases (e.g. Brugada syndrome, Long QT syndrome, conduction disease and cardiomyopathy), while genetic variation in the general population has been associated with differences in cardiac conduction and risk of arrhythmia through genome wide association studies. In this review we aim to give an overview of the current knowledge (and the gaps therein) on *SCN5A* and $Na_V1.5$.

INTRODUCTION

Voltage-gated sodium channels are responsible for the inward sodium current (I_{Na}) in excitable cells. As such, they induce a fast depolarization, thereby initiating an action potential[1]. In the heart, I_{Na} is crucial for fast impulse propagation through the tissue[2]. The main protein generating the cardiac sodium current is the pore-forming alpha subunit $Na_V1.5$, encoded by the gene *SCN5A*[3,4]. Since the cloning of this gene in 1992[3], a substantial amount of genetic and molecular biological information has been obtained regarding the role of this gene and its corresponding protein in health and disease. Mutations have been identified in families with inherited cardiac arrhythmia syndromes and genetic variation in the general population at the *SCN5A*-locus has been associated with electrocardiographic differences[5]. We here provide an overview of the current knowledge concerning the regulation and function of the gene *SCN5A* and its corresponding protein $Na_V1.5$ and discuss the association of gene mutations and common variants in relation to inherited cardiac diseases. For some of the pertinent topics of this review, excellent recent and highly detailed reviews are available (e.g. sodium channel interaction partners[4], posttranslational modifications[6] and splice regulation[7]). In these cases we here provide only a short summary and refer the reader to these reviews for further reading.

GENE *SCN5A*

Gene structure

SCN5A, i.e. sodium channel, voltage gated, type V alpha subunit, $Na_V1.5$, (ENSG00000183873, HGNC:10593, NCBI Gene ID: 6331), is part of a family of 10 genes encoding sodium channel alpha subunits. Of these $Na_V1.1$, $Na_V1.2$, $Na_V1.3$, $Na_V1.6$ and $Na_v2.1$ (Na_x) are the main sodium channels in the central nervous system; $Na_V1.7$, $Na_V1.8$ and $Na_V1.9$ in the peripheral nervous system; $Na_V1.4$ in skeletal muscle and $Na_V1.5$ which is encoded by *SCN5A* is the main sodium channel in the heart reviewed in[8]. *SCN5A* is a large highly conserved gene that is present from platypus to birds and human[8]. The gene *SCN5A* spans more than 100kb on human chromosome 3p22, and consists of 28 exons of which exon 1 and in part exon 2 forms the 5' untranslated region (5' UTR) and exon 28 the 3' untranslated region (3' UTR) of the RNA.

Expression pattern

SCN5A transcripts are mainly found in the heart, however transcript levels have been demonstrated in smooth muscle cells of the intestines[9] and in macrophages[10]. Also, the "neonatal" splice isoform of *SCN5A* (see below) is expressed in the central nervous system

and in certain types of cancer[8]. The functional role of *SCN5A* in these non-cardiac tissues is only slowly beginning to emerge. In the heart, *SCN5A* transcripts are highly abundant in working myocardium and conductive tissue, whereas the expression in the sinoatrial and atrioventricular nodes is relatively low[11]. Within the sinoatrial node, the central part is devoid of $Na_V1.5$ expression, while expression has been demonstrated in the periphery[12]. Across the ventricular wall, a transmural expression gradient exists, as reflected by higher expression of *SCN5A* in the subendocardial layer as compared to the subepicardium[11].

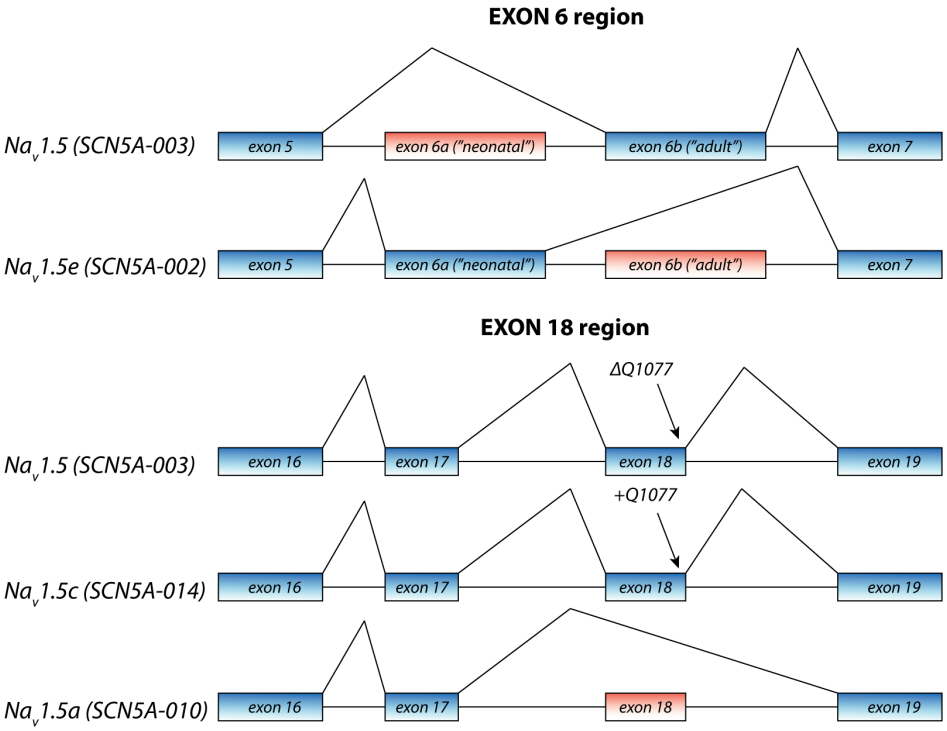

Figure 1. Illustrative scheme of splice variants of *SCN5A*. Annotations are according to Schroeter et al. (2010), with in parenthesis the ID-number as annotated in the Ensembl database. Alternative splicing mainly occurs at the region of exon 6 and exon 18.

Splice variants

More than 10 different splice isoforms have been described and are predicted based on sequence and cDNA. Many of these splice isoforms are differentially expressed in the heart and other tissues and are known to have different electrophysiological properties. However, not all splice isoforms have been studied in detail and discrepancies in the nomenclature between studies hamper easy comparison of the results. A clear comprehensive overview of the functional differences between the different splice isoforms is given in a recent review[7].

There are four major different *SCN5A* isoforms, of which SCN5A-003 (NM_000335) is the most abundant transcript in murine and human heart[7,13]. This transcript, also referred to as the "adult" isoform, replaces the "neonatal" SCN5A-001 (NM_001099404, $Na_V1.5e$) within a few days after birth in mice. The two isoforms differ in exon 6 (exon 6b in the adult and exon 6a in the neonatal isoform), resulting in a difference of 7 amino-acids[7,13]. SCN5A-001 is also abundantly expressed in neonatal murine brain and at low levels in the adult brain[14]. Functionally, these two isoforms differ significantly: compared to the adult isoform, the neonatal splice isoform exhibits a slower (de)-activation rate and a depolarized shift in voltage dependence of activation. Apart from these two isoforms, SCN5A-010 (no refseq ID) & SCN5A-014 (NM_198056) have been studied in some detail. SCN5A-010, which lacks exon 18, is expressed in the rat brain, heart and hippocampal progenitor cells. This splice isoform has not been found in human tissue as yet[7]. SCN5A-014 includes an additional CAG at the exon 17–18 splice boundary resulting in an additional glutamine at position 1077 (1077Q). This transcript is expressed in the heart albeit less abundantly than SCN5A-003 (ratio approximately 1:2)[7]. The transcript including 1077Q encodes channels that exhibit less I_{Na}[15]. Remarkably, certain polymorphisms demonstrate more pronounced effects in either the SCN5A-014 or the SCN5A-003 splice variant[16]. In addition to the coding splice variants described above, several different exon 1 variants have been described leading to alternative 5′ UTRs. It is likely that these differences in 5′ UTR play a role in translational regulation. Interestingly, the different exons 1 are partially species specific as they are different between the murine and human genome[17,18]. Functional consequences of these exon 1 splice variations remain to be elucidated.

Transcriptional regulation

Currently, three distinct promoter regions have been identified for *SCN5A*, corresponding to 4 different transcripts with alternative 5′ UTRs[17,19]. The original identified promoter consists of 2.8 kilobases and exceeds exon 1 and partially intron 1[19]. Several transcription factors that influence gene expression have been identified to date, including Forkhead Box O1 (Foxo1)[20,21], nuclear factor-κ B (NF-KappaB)[22] and TBX5[23,24]. Foxo1 and NF-KappaB are both involved in gene regulation upon oxidative stress, which is present for example during myocardial infarction or upon hypertrophic stimuli. Production of reactive oxygen species (ROS) leads to the nuclear translocation of these transcription factors and consequently inhibits transcription of *SCN5A* by directly binding the promoter region. TBX5, which plays a fundamental role during cardiac development, stimulates *SCN5A* expression in the adult cardiac conduction system by binding gene enhancer elements[23,24]. Apart from regulation through direct transcriptional control, regulation of *SCN5A* at the posttranscriptional level through microRNAs has been demonstrated, showing increased transcript and protein levels upon expression of miR-219[25].

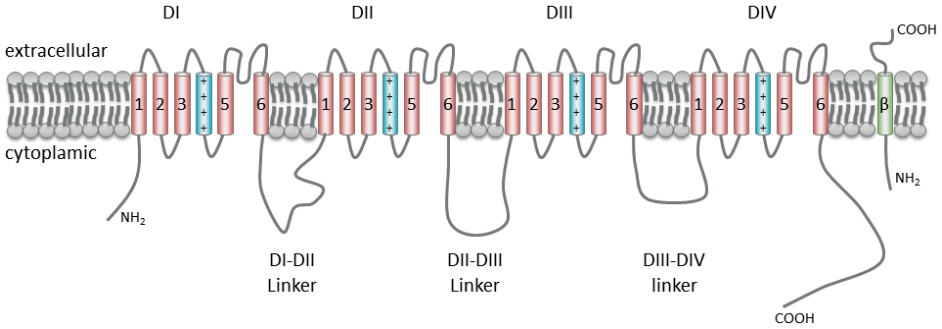

Figure 2. $Na_V1.5$ protein structure. The transmembrane segments S1–S6 are indicated by numbered cylinders; the fourth positively charged S segment, important in voltage sensing is depicted in blue. The transmembrane segment depicted in green resembles one of the beta-subunits.

STRUCTURE AND FUNCTION OF $NA_V1.5$

Protein structure

$Na_V1.5$ is a large transmembrane protein with 4 repetitive transmembrane domains (DI-DIV) with 6 transmembrane-spanning sections each (S1-S6). The four S5-S6 and intervening loop domains together are the central pore forming region of the α-subunit of the sodium channel, while the remaining S domains act as voltage sensors, in which the positively charged S4 domains play a crucial role (**Figure 2**)[8,26].

Biophysical properties

Similar to other voltage-gated sodium channels, $Na_V1.5$ exhibits different biophysical properties regarding its voltage and time-dependent conformational state (termed 'gating'), which determines whether the channel is opened (i.e. able to conduct Na^+-ions) or closed. During diastole, when the transmembrane electrical potential is around –85 mV, $Na_V1.5$-channels are in a closed state. As the membrane depolarizes upon a stimulus and a certain threshold is reached, channels become activated within 1 ms. This is mediated through a simultaneous outward movement of the S4 segments of all 4 transmembrane domains, resulting in the opening of the channel pore and, due to the electrochemical gradient, inward conductance of Na^+-ions. Consequently, a fast depolarization of the membrane is realized, reflecting phase 0 of the cardiac action potential (**Figure 3A**). Immediately upon depolarization, $Na_V1.5$ channels are closed through a process called 'fast inactivation'. Again the S4 segments, especially those from domain III and IV, are moved outwards, while the intracellular loop between domains III and IV functions as a 'lid' to close the channel pore. In the latter event, the amino acid sequence IFM (located at position 1488–1490) plays a key role[27,28]. Activation and inactivation of $Na_V1.5$ channels is voltage-dependent, as depicted in **Figure 3B**. In physiological conditions,

when inactivated, channels remain in closed state until the cell membrane is repolarized, allowing them to recover from inactivation and becoming available for activation again. While the membrane is still depolarized, $Na_V1.5$ channels undergo more conformational changes, reaching different states of inactivation, i.e. the 'intermediate-' and 'slow-inactivation' state. During the cardiac action potential, Na^+-channels never reach the full slow-inactivated state, as this happens only after a time frame of > 60 s[29]. The intermediate-inactivated can be reached during the action potential, albeit only by a small fraction of channels[30,31]. All these states require different times to recover during the repolarization phase: while recovery from fast-inactivation happens within 10 ms, Na-channels that reside in intermediate and slow-inactivation states require ~50 ms and > 5 s, respectively, until they are available for activation again. The exact biophysical processes underlying the different state of inactivation are not completely understood; here, important roles for different structural parts of the Na-channel (e.g. the pore, intracellular loops and voltage-sensor) have been suggested, pointing out different responsible mechanisms[32]. During the action potential, a very small fraction of sodium current persists and does not inactivate completely. This current is called 'sustained current', 'late current' or '$I_{Na,L}$' [33,34]. Finally, some channels may reactivate during the repolarizing phase of the action potential at a range of potentials where voltage dependent inactivation is not complete and shows overlap with activation, generating the so-called "window current" (**Figure 3A and B**)[35]. Both the window current and the sustained current can play important roles in genetic and acquired cardiac diseases, as discussed below.

Subunits and protein interaction partners

To date, a wide scale of interacting proteins regulating function or membrane expression of $Na_V1.5$ have been identified (extensively reviewed in Abriel, 2010). $Na_V1.5$ is part of a macromolecular complex in which different proteins interact and modify the trafficking, function, or structure of the channel. An important group of interacting proteins is formed by the 4 beta-subunits, transmembrane proteins encoded by the genes *SCN1B* to *SCN4B* that consist of only one transmembrane segment. While different voltage gated sodium channels do not exhibit a response to each beta-subunit, for the cardiac sodium channel $Na_V1.5$ a role has been assigned for each one of them. Although results on the direct exact physiological effects of beta-subunits on $Na_V1.5$-driven Na^+-current are conflicting[4,36], in general, beta-subunits increase currents by increase in trafficking of the channel to the cell surface or change in the intrinsic properties of the sodium channel, such as voltage dependence of (in)activation. Moreover, the interaction of $Na_V1.5$ with some other important interacting proteins is dependent on the presence of beta-subunits[36] . Finally, the crucial role of beta-subunits is highlighted by the fact that mutations in the genes *SCN1B* to *SCN4B* are implicated in different cardiac arrhythmia syndromes[37–40]. For example, *SCN1B*, which encodes the β1-subunit, has been associ-

ated with Brugada syndrome[38] conduction disease and atrial fibrillation. Apart from the beta-subunits, other proteins interacting and modulating function of $Na_V1.5$ have been identified, such as calmodulin, calmodulin kinase II δc, ankyrin-G and plakophilin-2, of which some have also been linked to genetic and acquired cardiac diseases[41,42]. These interactions as well as information regarding posttranslational modifications of the cardiac sodium channel have been reviewed in detail[46].

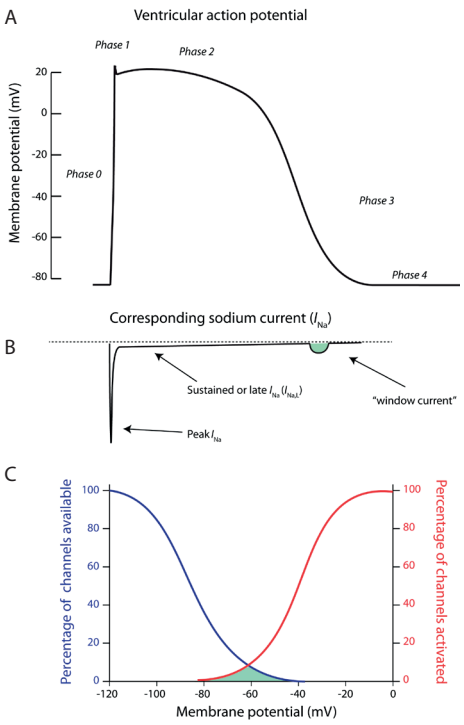

Figure 3. $Na_v1.5$-driven I_{Na} in normal conditions. **A**. The ventricular action potential as a function of time and **B**. the corresponding I_{Na} in the physiological situation. The window current is depicted in green. **C**. Illustration of the percentage of available (blue) and activated (red) channels as a function of the membrane potential. The window current (indicated in green) is formed at potentials at which inactivation and activation are overlapping.

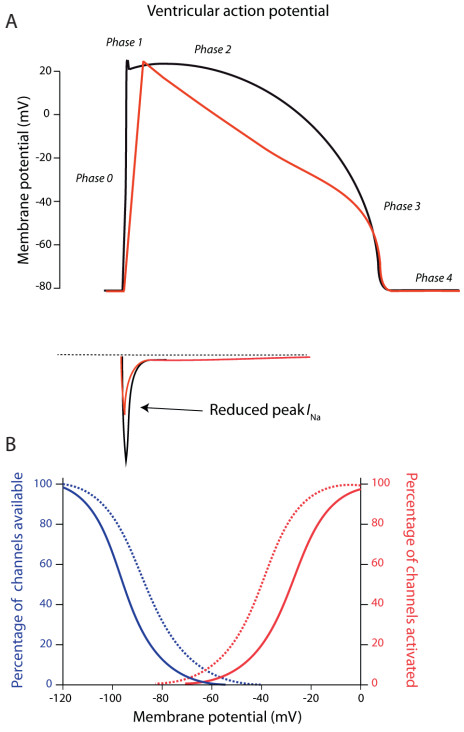

Figure 4. Reduced function of I_{Na} as a consequence of loss-of-function mutation in *SCN5A*. **A**. Decreased peak I_{Na} lowers the upstroke velocity of the action potential (red trace). **B**. Shifts in voltage dependence of (in)activation (dashed lines) that result in loss-of-function of I_{Na}. Apart frommutations that lower the amount of channels at the membrane or reduce conductivity of the channel, this phenomenon forms an alternative mechanism for reduced functionality.

GENETICS

SCN5A mutations: association with disease

Mutations in *SCN5A* can disrupt proper function of $Na_V1.5$ and as such lead to different, mainly cardiac, diseases. Both loss- and gain of-function mutations are described, while occasionally a mutation results in functional channels with aspects of both, leading to a disease with overlapping phenotypes. Most commonly, pathogenic *SCN5A* mutations show an autosomal dominant inheritance pattern, with incomplete penetrance, but also recessive forms with homozygous or compound heterozygous mutations are described[31,45–48]. Interestingly, the observed phenotypes in *SCN5A* mutation carriers are highly diverse. This diversity could in part be explained by the fact that different biophysical aspects of the channel (e.g. voltage dependence of (in)-activation, conductivity, $I_{Na,L}$) could be affected by a mutation, leading to both loss- and gain-of-function (**Figure 4 and 5**, respectively). However, even within families, different clinical phenotypes can be observed, suggesting important roles for environmental and other (common) genetic factors[43,49,50]. To date, loss-of-function mutations have been associated with Brugada syndrome (BrS)[43,50,51] progressive cardiac conduction disease (Lev-Lenègre disease)[52,53], dilated cardiomyopathy (DCM)[54–57], sick sinus syndrome (SSS)[58,59] and atrial fibrillation (AF)[60]. Mutations resulting in a gain-of-function are causal for Long QT syndrome (LQTS) type 3 [43,61] and are also more recently implicated in Multifocal ectopic Purkinje related premature contractions (MEPPC)[57,62,63]. Some gain-of-function mutations are also associated with AF and DCM[56].

Brugada syndrome

Brugada syndrome (BrS) is a familial arrhythmia syndrome characterized by ST-elevations in the right precordial leads on the ECG. Patients with Brugada syndrome are at substantial risk for development of tachyarrhythmia and sudden cardiac death. While originally described as a disease in which no cardiac structural defects are present, different reports have pointed out subtle microscopic structural abnormalities in hearts from BrS patients, specifically fibrosis [64]. Also, in many cases BrS patient present with cardiac conduction slowing. The exact mechanism underlying the ECG abnormalities and the arrhythmia are still controversial [65]. However, given that ~25% of BrS patients possess a loss-of-function mutation in *SCN5A* and that the typical ECG features can be evoked by challenge with sodium channel blockers (e.g. ajmaline), the cardiac sodium channel plays an important role in this disease [66]. However, apart from incomplete penetrance ofmutations, presence of the disease in absence of the familial *SCN5A* mutation is not uncommon, indicating the genetic complexity of the disease [67].

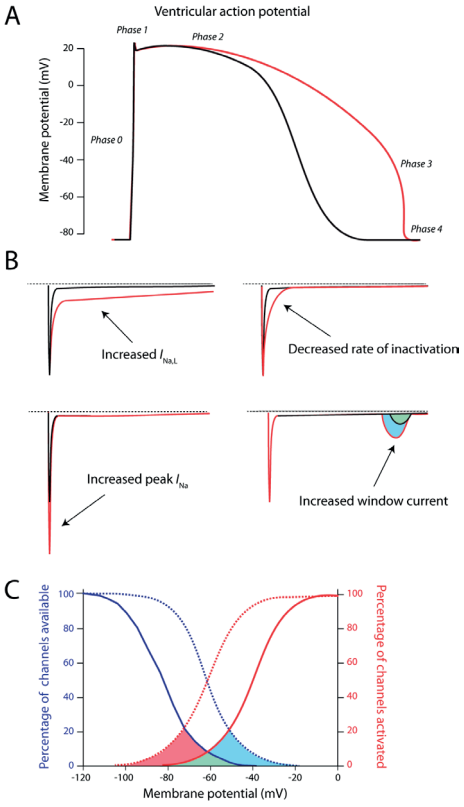

Figure 5. Functional effects of gain-of-function mutations in *SCN5A*. **A**. Consequences of gain-of-function on the ventricular action potential. Due to an increased net influx of Na^+-ions, action potential duration is increased (red trace), which can evoke arrhythmic events. **B**. Different mechanisms that may be responsible for gain-of-function in I_{Na}, i.e. increased late current ($I_{Na,L}$), increased peak I_{Na}, decreased rate of inactivation and increased window current. Most commonly, an increase in sustained current ($I_{Na,L}$) is observed. **C**. Shifts in voltage dependence of (in)activation (dashed lines) that lead to an increased window current, as illustrated by the red and blue areas under the curve.

Progressive cardiac conduction disease (Lev-Lenègre disease)

While the mechanism of loss of function mutations underlying BrS is still heavily debated, the role of *SCN5A* mutations in progressive cardiac conduction disease, or Lev-Lenègre disease, is more clear, given the important role of $Na_v1.5$ in the specialized cardiac conduction system [11]. Patients with Lev-Lenègre disease present usually at more advanced age with widened QRS complexes on the ECG, in combination with a left- or right-bundle-branch block that can eventually culminate into complete atrioventricular block. Although it is a very common cardiac condition, Lev-Lenègre disease shows a familial inheritance only sporadically. Mutations in *SCN5A* as a cause of Lev-Lenègre disease were first described in 1999 [52], after which more reports followed [53].

Sick sinus syndrome (SSS)

Sick sinus syndrome (SSS) is a disease characterized by malfunction of the sinus node in which patients exhibit sinus bradycardia, sinus arrest and reduced chronotropic response [58,68,69]. Although the disease usually manifests at later age, mostly due to structural defects related to fibrosis or ischemia, families in which SSS manifests at younger

age and inherits according to Mendelian patterns, are described. One of the genes that has been linked to SSS is *SCN5A* [31,58–60,70]. Since expression of *SCN5A* is low in central sinus nodal cells and sodium channels are mostly inactivated during the relatively positive diastolic potential, mutations in *SCN5A* have only small impact on individual primary pacemaker cells. However, in the periphery of the sinus node, in which *SCN5A* is expressed loss-of-function mutations can affect the amount of 'window' current during the diastolic phase, thereby lowering the speed of diastolic depolarization at the single cell level [68,69]. The role of $Na_V1.5$ in sinus node cells has been highlighted in mouse models, demonstrating sinus node dysfunction in *Scn5a* knockout mice[71] and slowed sinus node conduction upon $Na_V1.5$ blockade[12]. Considering the connections between sinus node and atrium, there is less inward current in the sinus node counteracting the more negative diastolic potential of atrial cells, resulting in hyperpolarization of the central sinus node cells that leads to slowing of the firing rate. Moreover, reduced excitability of atrial cells due to the mutation can lead to exit block, a common feature in SSS[69].

Atrial fibrillation

Atrial fibrillation (AF) is the most common arrhythmia syndrome in the Western World[72]. During AF, atria exhibit involuntary contractions rather than the simultaneous single contraction normally occurring after depolarization of the atria, which is the result of a continuous chaotic and unorganized electrical activity. In general, the disease occurs in the context of structural heart disease. In the absence of any structural abnormalities, especially when it arises in relatively young people, and when a familial pattern is observed, a genetic cause is suspected, however the genetic causes of AF remain largely elusive thus far[73]. Emerging evidence suggests a possible link between mutations in *SCN5A* and familial AF: (i) a high prevalence of *SCN5A* mutations that co-segregated among family members was noted in AF patients [74,75]; (ii) there is a high degree of overlap between atrial fibrillation and other diseases associated with *SCN5A* mutations, i.e. BrS, LQT3 and conduction disease[75–77]; (iii) the common polymorphism $Na_V1.5$-H558R, that results in a slight reduction of function, is more prevalent among AF patients than in controls [78,79]. As both gain- and loss-of-function mutations have been described, the possible underlying pathogenic mechanism remains unclear.

Long QT syndrome type III

The first mutation described for *SCN5A* was ΔKPQ and was linked to congenital Long QT syndrome type III (LQT3). Patients with LQT syndrome exhibit prolonged QT-intervals at the ECG, which is reflected by an increased action potential duration at the cellular level. Moreover, patients are at increased risk for the development of polymorphic ventricular tachycardia, specifically torsade de pointes[61]. From all LQTS patients, 5–10% carry a mutation in *SCN5A*, whereas 90% of the patients possess a mutation in the potassium

channel encoding genes *KCNQ1* and *KCNH2* (manifesting as LQT1 and LQT2, respectively)[80]. Gain-of-function mutations in *SCN5A* that result in LQT3 usually impact on the inactivation characteristics of the sodium channel, which is either slowed or incomplete. Due to failure to inactivate completely, the late component of the sodium current is increased, leading to a persistent inward current during the plateau phase of the action potential and subsequently a prolongation. An alternative mechanism is a shift in voltage dependence of inactivation, resulting in an increase in window current. Finally, an increased rate of recovery from inactivation can result in an increased rate of channel reopening during the repolarization phase, as illustrated for the mutation I1768V[81].

Multifocal Ectopic Purkinje-Related Premature Contractions

Recently, the mutation R222Q in *SCN5A* was identified in different unrelated families in which mutation carriers exhibited frequent premature ventricular complexes arising from the Purkinje system, leading to ventricular tachycardia and sudden death in some cases [57,62,82]. Moreover, this arrhythmia syndrome, annotated as Multifocal Ectopic Purkinje-Related Premature Contractions (MEPPC), was associated with dilated cardiomyopathy, probably secondary to the arrhythmias. Functional studies revealed that the R222Q mutation exhibits a negative shift in both voltage dependence of activation and inactivation, indicating both a gain- and loss of function, however the net effect gives rises to an increase and shift in window current. Modeling studies performed in that study showed that the mutation affects the action potential of Purkinje cells , i.e. repolarization is delayed, while ventricular cells are not affected by the mutation, in alignment with the origin of the premature contractions. Also, the effects were more profound at rest, a phenomenon also observed in the mutation carriers. Apart from R222Q, a different study identified another mutation located in the same region, R225P, which generates a similar phenotype with similar biophysical changes. While premature ventricular complexes are mainly present at rest in MEPPC cases, a recent study has linked a variant in *SCN5A* to a family with patients experiencing a high frequency of exercise-induced premature beats[63]. Also this variant (I141V) exhibited an increased window current, similar to the R222Q mutation.

Dilated cardiomyopathy (DCM)

The association of *SCN5A* mutations and dilated cardiomyopathy (DCM)[54–57,83], a structural heart disease characterized by dilated chambers, pump failure and a high incidence of arrhythmia, is possibly the most intriguing and surprising one. To date, the mechanism underlying the disease in the case of *SCN5A* mutations is mainly speculative. This has been complicated by the fact that the identified mutations show a high degree of functional divergence (both gain- and loss-of functions, different types of biophysical changes), and the disease is highly heterogeneous [84]. Several pathophysiological mecha-

nisms have been proposed. First, DCM could develop secondary to frequent arrhythmia or sinus node dysfunction, as is observed with MEPPC[85,86]. In the case of sinus node dysfunction, which is described in several patients with DCM and *SCN5A*-mutations, low heart rate can lead to remodeling and hypertrophy, as described in the dog model of chronic atrioventricular block[87]. In the second hypothesis, it is proposed that increased window current or persistent current causes disturbance in Na^+ homeostasis, which in turn leads to alterations in intracellular Ca^{2+} and pH, through the Na^+/Ca^{2+}- and Na^+/H^+-exchanger, respectively. It should be noted however that in LQT3 patients persistent Na^+-influx does not lead to such a pronounced structural phenotype. Recent studies demonstrated the presence of a proton-based leak current[88,89] caused by the mutations R225W, R222Q and R219H, which could alternatively induce cellular acidification and secondary to that cellular remodeling. Interestingly, all these mutations are located in the same domain while generating diverging biophysical effects[88,89]. Finally, the mechanism by which *SCN5A*-mutations cause DCM could be solely non-electrical. At the intercalated disks of cardiomyocytes, $Na_v1.5$ is part of a macromolecular complex [90] that includes structural proteins, and it is conceivable that disruption of these interactions can cause downstream structural problems.

SCN5A variations in the general population

Genetic variations in *SCN5A*, i.e. single nucleotide polymorphisms (SNPs) which are present at relatively high frequencies within the general population, have been described in both coding and noncoding regions of the gene. Regarding non-coding region variants, several have been identified in the promoter region and are known to alter transcriptional activity of *SCN5A*[19]. As such, these SNPs affect conduction in healthy and diseased patients and arrhythmia susceptibility in *SCN5A* mutation carriers[81]. As for variants in the coding region, several have been linked to arrhythmia syndromes and are studied functionally. For example H558R, present at a allele frequency of 20–30%, is known to aggravate or attenuate the effects of disease causing mutations[91,92] while presence in a wildtype channel reduces current, depending on the presence of the splice variant that includes Q1077[15]. Other studied polymorphisms include S1102Y[93] (10% in Blacks) and R1193Q (8% in Asians)[94], which have been linked to BrS and LQT3, respectively.

Rare variants: evidence of pathogenicity

Most phenotypes associated with *SCN5A* mutations have been identified through candidate gene studies rather than unbiased, genome wide studies such as linkage analysis in large pedigrees. This is mainly a result of the lack of large enough families to perform such studies. The evidence of involvement of *SCN5A* mutations in the phenotypes described above thus depends on the large enrichment of these kind of mutations in the patient cohorts (5–10% in LQTS3, 25% in BrS) and on segregation testing within families

in addition to functional characterization of the identified mutations. However, ascribing pathogenicity to a rare variant of unknown significance (VUS) identified in *SCN5A* in a patient is not straight forward. With the advent of exome sequencing, large panels of individuals have now been sequenced for variants in *SCN5A*, the results of which are publicly available through online databases such as the Exome Variant Server[95] and ExAC[96]. Inspection of these databases shows that between 2–7% of the individuals screened in these cohorts carry rare (population frequency < 1%) protein altering variants in *SCN5A* **(Table 1)**. The majority of these are missense mutations, which could in theory lead to gain and loss of function phenotypes. Given the low prevalence of *SCN5A* mutation associated diseases it is highly improbable that all these VUSs significantly contribute to disease, although subtle effects of variations could modify disease susceptibility. In clinical practice this means that ascribing pathogenicity to *SCN5A* missense VUS identified in individual patients is not straightforward, especially when no affected relatives are available for segregation testing[97,98]. In contrast, variants that dramatically alter protein structure (splice, stop gain and frameshift) are very rare in these cohorts (**Table 1**), indicating that these variants are most likely pathogenic when encountered in patients with a loss of function phenotype. In addition to the distinction between dramatic change and missense change, information from *in silico* prediction tools and the affected protein domain can be informative. A combination of all the available information helps to distinguish true pathogenic variants from background genetic noise. Unfortunately, even then many variants remain in the limbo as VUS[99].

Table 1. SCN5A genetic variation in the general population

Type of mutation	frequency in EVS (n> 6500)	frequency in ExAC n> 120,000
missense	1.89%	7.40%
splice	0.02%	0.01%
stopgain	-	0.01%
frameshift	-	0.01%
in frame deletion	-	0.02%
Total	**1.91%**	**7.45%**

Common variants: Genome Wide Association Studies

Genome Wide Association Studies (GWAS) have employed common genetic variation to identify genetic loci associated with variability in phenotypic traits. In the cardiovascular field this powerful technique has been used to detect genomic loci involved in variation in electrocardiographic parameters (i.e. PR-, QR- and QTc-interval duration) in the general population (reviewed in[100]. The rationale behind this technique is that common genetic variation present in the general population can influence cardiac conduction in non-diseased individuals. These studies have been tremendously successful

in identifying novel loci that impact on cardiac conduction. Interestingly, these studies consistently identified the *SCN5A* and *SCN10A* genomic region on chromosome 3 to be associated with variation in QTc-interval, QRS duration and PR-interval[100]. These results are consistent with the notion that common variants at loci implicated in "Mendelian" disease can confer smaller effects within the general population on related or intermediate phenotypes such as ECG parameters. Furthermore the *SCN5A* locus has been implicated in the risk of sudden cardiac death in a candidate gene study in the general population[101]. Whether the effects of the independent GWAS signals in *SCN5A* and *SCN10A* (the genes are juxtaposed at 60 kb from each other at chr3p22) are mediated through *SCN5A* alone[24] or partially to the $Na_v1.8$ encoding gene *SCN10A* as well[102,103] is still a matter of debate. Of note, transcript levels of *SCN10A* did not exceed the detection limit of RNA sequencing in ventricular tissues[104], suggesting a limited role of this gene in cardiomyocytes.

$NA_V1.5$ AS A PHARMACOLOGICAL TARGET

The cardiac sodium channel $Na_V1.5$ has since long been a common target in the pharmacological treatment of arrhythmic events. Classically, sodium channel blockers that block the peak sodium current are classified as Class I anti-arrhythmic agents and further subdivided in classes IA, IB and IC, depending on their ability to change the length of the cardiac action potential[105]. The mode of action of these blockers may depend on the biophysical state of the Na_+-channel: while some blockers bind channels in activated state (open-state block), others block when the channels are in inactivated state (closed-state block). This paradigm is described in the "modulated receptor hypothesis"[106,107]. While the blockade of sodium channels can stop reentrant wavefronts by reducing excitability of cardiomyocytes and increasing refractory period, the same mechanism may actually exert opposing effects and can evoke arrhythmia in specific situations. This however depends on the intrinsic parameters of the drug (use-dependency, dissociation and binding rates, concomitant block of K^+ and Ca^{2+} channels) and on the condition during which the drug is applied (e.g. ischemia). Use of sodium channel blockers is among others indicated in patients with ventricular reentrant tachyarrhythmia in the setting of cardiac ischemia and in patients with atrial fibrillation in absence of structural heart disease[106].

Apart from acting on peak sodium current, sodium channel blockers also impact on the late component of the sodium current ($I_{Na,L}$) to different extents. Drugs that inhibit mainly $I_{Na,L}$ are of potential clinical interest as an increase in $I_{Na,L}$ is involved in different conditions. Ranolazine, the most selective $I_{Na,L}$ that is currently in clinical use, is approved by the FDA for the treatment of angina pectoris. During ischemia, in which $I_{Na,L}$ is en-

hanced, ranolazine reduces intracellular Ca^{2+} concentration indirectly through the Na^+/Ca^{2+}-exchanger, and thereby cardiac workload. As an antiarrhythmic agent, ranolazine is especially of interest in the treatment of LQT3, where $I_{Na,L}$ directly prolongs action potential duration and causes QT prolongation, as proven by several preclinical and clinical studies[108,109]. Furthermore, different studies suggest that ranolazine is a potential candidate in treating atrial fibrillation[110–112]. Given that ranolazine can exert unwanted effects by blocking repolarizing potassium currents[113], possibly evoking drug-induced Long QT-syndrome, more selective drugs are currently under development[114].

NON-CANONICAL ROLES OF $NA_V1.5$

Apart from the role of voltage-gated sodium channels in excitable cells, there is emerging evidence for a function of these channels in non-excitable cells, in particular in different type of cancer cells. This also holds for $Na_V1.5$ of which the "neonatal" isoform is expressed and functional in among others breast cancer cells, colon cancer and brain astrocytoma[115–118]. In these cells, $Na_V1.5$ function is associated with increased invasiveness and the development of metastases[119]. In breast cancer cells, $Na_V1.5$ co-localizes with the Na^+/H^+-exchanger (NHE-1)[115], where it affects activity of NHE-1 allosterically, leading to local extracellular acidification. This acidification results in an increased activity of proteolytic enzymes (cathepsins) that are pH-sensitive and responsible for breakdown of extracellular matrix. Influx of Na^+-ions is also of importance, as blocking the channels in cancer cells directly with sodium current blockers affects the degree of invasion[117,118]. Recent studies have identified $Na_V1.5$ in endosomes of macrophages present within lesions of the neurological disease multiple sclerosis[10]. Here the channels contribute to phagocytosis and pH-regulation within the endosome. It is suggested that also for this disease, targeting $Na_V1.5$ would form a putative therapeutic approach.

CONCLUDING REMARKS

Although many aspects regarding the gene SCN5A and its protein Nav1.5 have been clarified, many avenues are still to be explored. The divergent phenotype that is associated with SCN5A mutations, ranging from arrhythmia to dilated cardiomyopathy, plus the incomplete penetrance of mutations that even affect channel properties severely, remain interesting, yet unexplained phenomenons. It is tempting to speculate that other factors, not related to the gene SCN5A, are important in determining the eventual clinical phenotype. In line with this suggestion are the association studies that have linked common genetic variations in the SCN5A locus to changes in conduction parameters.

Albeit such variations do not cause disease per se, they can contribute to significant conduction slowing in conjunction with other factors. The exact relevance and impact of common (and uncommon) variances in the general population, however, remain elusive. The development of novel disease models, such as cardiomyocytes derived from human induced pluripotent stem cells44, could potentially result in novel insights regarding these issues.

REFERENCES

1. Weidmann, S. The effect of the cardiac membrane potential on the rapid availability of the sodium-carrying system. *J. Physiol.* **127,** 213–224 (1955).
2. Buchanan, J. W., Saito, T. & Gettes, L. S. The effects of antiarrhythmic drugs, stimulation frequency, and potassium-induced resting membrane potential changes on conduction velocity and dV/dtmax in guinea pig myocardium. *Circ. Res.* **56,** 696–703 (1985).
3. Gellens, M. E. *et al.* Primary structure and functional expression of the human cardiac tetrodotoxin-insensitive voltage-dependent sodium channel. *Proc. Natl. Acad. Sci. U. S. A.* **89,** 554–558 (1992).
4. Abriel, H. Cardiac sodium channel $Na_v1.5$ and interacting proteins: Physiology and pathophysiology. *J. Mol. Cell. Cardiol.* **48,** 2–11 (2010).
5. Remme, C. A. Cardiac sodium channelopathy associated with *SCN5A* mutations: electrophysiological, molecular and genetic aspects. *J. Physiol.* **591,** 4099–116 (2013).
6. Marionneau, C. & Abriel, H. Regulation of the cardiac Na^+ channel $Na_V1.5$ by post-translational modifications. *J. Mol. Cell. Cardiol.* **82,** 36–47 (2015).
7. Schroeter, A. *et al.* Structure and function of splice variants of the cardiac voltage-gated sodium channel $Na_V1.5$. *J. Mol. Cell. Cardiol.* **49,** 16–24 (2010).
8. Catterall, W. a. Sodium channels, inherited epilepsy, and antiepileptic drugs. *Annu. Rev. Pharmacol. Toxicol.* **54,** 317–38 (2014).
9. Holm, A. N. *et al.* Sodium current in human jejunal circular smooth muscle cells. *Gastroenterology* **122,** 178–187 (2002).
10. Black, J. A., Newcombe, J. & Waxman, S. G. $Na_v1.5$ sodium channels in macrophages in multiple sclerosis lesions. *Mult. Scler.* **19,** 532–42 (2013).
11. Remme, C. A. *et al.* The cardiac sodium channel displays differential distribution in the conduction system and transmural heterogeneity in the murine ventricular myocardium. *Basic Res Cardiol* **104,** 511–522 (2009).
12. Lei, M. *et al.* Requirement of neuronal- and cardiac-type sodium channels for murine sinoatrial node pacemaking. *J. Physiol.* **559,** 835–848 (2004).
13. Onkal, R. *et al.* Alternative splicing of $Na_V1.5$: An electrophysiological comparison of 'neonatal' and 'adult' isoforms and critical involvement of a lysine residue. *J. Cell. Physiol.* **216,** 716–726 (2008).
14. Chioni, A. M. *et al.* A novel polyclonal antibody specific for the $Na_V1.5$ voltage-gated Na^+ channel 'neonatal' splice form. *J. Neurosci. Methods* **147,** 88–98 (2005).
15. Makielski, J. C. *et al.* A Ubiquitous Splice Variant and a Common Polymorphism Affect Heterologous Expression of Recombinant Human SCN5A Heart Sodium Channels. *Circ. Res.* **93,** 821–828 (2003).
16. Tan, B. H. *et al.* Common human *SCN5A* polymorphisms have altered electrophysiology when expressed in Q1077 splice variants. *Hear. Rhythm* **2,** 741–747 (2005).
17. van Stuijvenberg, L. *et al.* Alternative promoter usage and splicing of the human *SCN5A* gene contribute to transcript heterogeneity. *DNA Cell Biol.* **29,** 577–87 (2010).
18. Shang, L. L. & Dudley, S. C. Tandem promoters and developmentally regulated 5'- and 3'-mRNA untranslated regions of the mouse Scn5a cardiac sodium channel. *J. Biol. Chem.* **280,** 933–940 (2005).
19. Yang, P., Kupershmidt, S. & Roden, D. M. Cloning and initial characterization of the human cardiac sodium channel (*SCN5A*) promoter. *Cardiovasc. Res.* **61,** 56–65 (2004).

20. Cai, B. *et al.* Deletion of FoxO1 leads to shortening of QRS by increasing Na^+ channel activity through enhanced expression of both cardiac $Na_V1.5$ and β3 subunit. *J. Mol. Cell. Cardiol.* **74,** 297–306 (2014).
21. Mao, W. *et al.* Reactive oxygen species suppress cardiac $Na_V1.5$ expression through Foxo1. *PLoS One* **7,** e32738 (2012).
22. Shang, L. L. *et al.* NF-kappaB-dependent transcriptional regulation of the cardiac scn5a sodium channel by angiotensin II. *Am. J. Physiol. Cell Physiol.* **294,** C372–9 (2008).
23. Arnolds, D. E. *et al.* TBX5 drives *Scn5a* expression to regulate cardiac conduction system function. *J. Clin. Invest.* **122,** 2509–18 (2012).
24. Van Den Boogaard, M. *et al.* Genetic variation in T-box binding element functionally affects *SCN5A/SCN10A* enhancer. *J. Clin. Invest.* **122,** 2519–30 (2012).
25. Daimi, H. *et al.* Regulation of *SCN5A* by microRNAs: miR-219 modulates *SCN5A* transcript expression and the effects of flecainide intoxication in mice. *Heart Rhythm* **12,** 1333–1342 (2015).
26. Chen-Izu, Y. *et al.* Na^+ channel function, regulation, structure, trafficking and sequestration. *J. Physiol.* **593,** 1347–1360 (2015).
27. West, J. W. *et al.* A cluster of hydrophobic amino acid residues required for fast Na^+-channel inactivation. *Proc. Natl. Acad. Sci. U. S. A.* **89,** 10910–10914 (1992).
28. Kellenberger, S., Scheuer, T. & Catterall, W. a. Movement of the Na^+ channel inactivation gate during inactivation. *J. Biol. Chem.* **271,** 30971–30979 (1996).
29. O'Reilly, J. P., Wang, S. Y., Kallen, R. G. & Wang, G. K. Comparison of slow inactivation in human heart and rat skeletal muscle Na^+ channel chimaeras. *J. Physiol.* **515 (Pt 1,** 61–73 (1999).
30. Nuss, H. B., Chiamvimonvat, N., Pérez-García, M. T., Tomaselli, G. F. & Marbán, E. Functional association of the beta 1 subunit with human cardiac (hH1) and rat skeletal muscle (mu 1) sodium channel alpha subunits expressed in Xenopus oocytes. *J. Gen. Physiol.* **106,** 1171–1191 (1995).
31. Veldkamp, M. W. *et al.* Contribution of sodium channel mutations to bradycardia and sinus node dysfunction in LQT3 families. *Circ. Res.* **92,** 976–983 (2003).
32. Groome, J. R. Voltage Gated Sodium Channels. *Handb. Exp. Pharmacol.* **221,** 7–32 (2014).
33. Sakmann, B. F., Spindler, a J., Bryant, S. M., Linz, K. W. & Noble, D. Distribution of a persistent sodium current across the ventricular wall in guinea pigs. *Circ. Res.* **87,** 910–914 (2000).
34. Maltsev, V. a *et al.* Novel, ultraslow inactivating sodium current in human ventricular cardiomyocytes. *Circulation* **98,** 2545–2552 (1998).
35. Attwell, D., Cohen, I., Eisner, D., Ohba, M. & Ojeda, C. The steady state TTX-sensitive ('window') sodium current in cardiac Purkinje fibres. *Pflügers Arch. Eur. J. Physiol.* **379,** 137–142 (1979).
36. Meadows, L. S. & Isom, L. L. Sodium channels as macromolecular complexes: implications for inherited arrhythmia syndromes. *Cardiovasc Res* **67,** 448–458 (2005).
37. Watanabe, H. *et al.* Flecainide prevents catecholaminergic polymorphic ventricular tachycardia in mice and humans. *Nat Med* **15,** 380–383 (2009).
38. Watanabe, H. *et al.* Sodium channel beta1 subunit mutations associated with Brugada syndrome and cardiac conduction disease in humans. *J Clin Invest* **118,** 2260–2268 (2008).
39. Hu, D. *et al.* A mutation in the beta-3 subunit of the cardiac sodium channel associated with brugada ECG phenotype. *Circ. Cardiovasc. Genet.* **2,** 270–278 (2009).
40. Medeiros-Domingo, A. *et al. SCN4B*-encoded sodium channel beta4 subunit in congenital long-QT syndrome. *Circulation* **116,** 134–42 (2007).
41. Cerrone, M. *et al.* Missense mutations in plakophilin-2 cause sodium current deficit and associate with a Brugada syndrome phenotype. *Circulation* **129,** 1092–103 (2014).

42. Herren, A. W., Bers, D. M. & Grandi, E. Post-translational modifications of the cardiac Na channel: contribution of CaMKII-dependent phosphorylation to acquired arrhythmias. *Am. J. Physiol. Heart Circ. Physiol.* **305,** H431–45 (2013).
43. Remme, C. A. *et al.* Overlap syndrome of cardiac sodium channel disease in mice carrying the equivalent mutation of human *SCN5A*-1795insD. *Circulation* **114,** 2584–2594 (2006).
44. Davis, R. P. *et al.* Cardiomyocytes Derived from Pluripotent Stem Cells Recapitulate Electrophysiological Characteristics of an Overlap Syndrome of Cardiac Sodium Channel Disease. *Circulation* **125,** 31–71 (2012).
45. Lupoglazoff, J. M. *et al.* Homozygous mutation of the *SCN5A* gene responsible for congenital long QT syndrome with 2/1 atrioventricular block. Archives des maladies du coeur et des vaisseaux **95,** 440–446 (2002).
46. Neu, A. *et al.* A homozygous *SCN5A* mutation in a severe, recessive type of cardiac conduction disease. *Hum. Mutat.* **31,** 1609–1621 (2010).
47. Lopez, K. N., Decker, J. a., Friedman, R. a. & Kim, J. J. Homozygous mutation in *SCN5A* associated with atrial quiescence, recalcitrant arrhythmias, and poor capture thresholds. *Hear. Rhythm* **8,** 471–473 (2011).
48. Frigo, G. *et al.* Homozygous *SCN5A* mutation in Brugada syndrome with monomorphic ventricular tachycardia and structural heart abnormalities. *Europace* **9,** 391–397 (2007).
49. Nakajima, S. *et al.* A novel *SCN5A* mutation demonstrating a variety of clinical phenotypes in familial sick sinus syndrome. *Intern. Med.* **52,** 1805–8 (2013).
50. Bezzina, C. *et al.* A single Na^+ channel mutation causing both long-QT and Brugada syndromes. *Circ. Res.* **85,** 1206–1213 (1999).
51. Chen, Q. *et al.* Genetic basis and molecular mechanism for idiopathic ventricular fibrillation. *Nature* **392,** 293–296 (1998).
52. Schott, J. J. *et al.* Cardiac conduction defects associate with mutations in *SCN5A*. *Nat Genet* **23,** 20–21 (1999).
53. Tan, H. L. *et al.* A sodium-channel mutation causes isolated cardiac conduction disease. *Nature* **409,** 1043–1047 (2001).
54. McNair, W. P. *et al. SCN5A* mutation associated with dilated cardiomyopathy, conduction disorder, and arrhythmia. *Circulation* **110,** 2163–2167 (2004).
55. Groenewegen, W. a & Wilde, a a M. Letter regarding article by McNair et al, '*SCN5A* mutation associated with dilated cardiomyopathy, conduction disorder, and arrhythmia'. *Circulation* **112,** e9; author reply e9–e10 (2005).
56. Olson, T. M. *et al.* Sodium channel mutations and susceptibility to heart failure and atrial fibrillation. *JAMA* **293,** 447–454 (2005).
57. Laurent, G. *et al.* Multifocal ectopic Purkinje-related premature contractions: a new *SCN5A*-related cardiac channelopathy. *J Am Coll Cardiol* **60,** 144–156 (2012).
58. Benson, D. W. *et al.* Congenital sick sinus syndrome caused by recessive mutations in the cardiac sodium channel gene (*SCN5A*). *J. Clin. Invest.* **112,** 1019–1028 (2003).
59. Smits, J. P. P. *et al.* A mutation in the human cardiac sodium channel (E161K) contributes to sick sinus syndrome, conduction disease and Brugada syndrome in two families. *J. Mol. Cell. Cardiol.* **38,** 969–81 (2005).
60. Makiyama, T. *et al.* A novel *SCN5A* gain-of-function mutation M1875T associated with familial atrial fibrillation. *J. Am. Coll. Cardiol.* **52,** 1326–34 (2008).
61. Wang, Q. *et al. SCN5A* mutations associated with an inherited cardiac arrhythmia, long QT syndrome. *Cell* **80,** 805–811 (1995).

62. Mann, S. A. *et al.* R222Q *SCN5A* mutation is associated with reversible ventricular ectopy and dilated cardiomyopathy. *J. Am. Coll. Cardiol.* **60,** 1566–1573 (2012).
63. Swan, H. *et al.* Gain-of-Function Mutation of the *SCN5A* Gene Causes Exercise-Induced Polymorphic Ventricular Arrhythmias. *Circ. Cardiovasc. Genet.* **7,** 771–781 (2014).
64. Coronel, R. *et al.* Right ventricular fibrosis and conduction delay in a patient with clinical signs of Brugada syndrome: a combined electrophysiological, genetic, histopathologic, and computational study. *Circulation* **112,** 2769–77 (2005).
65. Hoogendijk, M. G., Potse, M., Vinet, A., de Bakker, J. M. T. & Coronel, R. ST segment elevation by current-to-load mismatch: an experimental and computational study. *Heart Rhythm* **8,** 111–8 (2011).
66. Lippi, G., Montagnana, M., Meschi, T., Comelli, I. & Cervellin, G. Genetic and clinical aspects of Brugada syndrome: an update. *Adv. Clin. Chem.* **56,** 197–208 (2012).
67. Probst, V. *et al. SCN5A* Mutations and the role of genetic background in the pathophysiology of Brugada syndrome. *Circ. Cardiovasc. Genet.* **2,** 552–557 (2009).
68. Abe, K. *et al.* Sodium channelopathy underlying familial sick sinus syndrome with early onset and predominantly male characteristics. *Circ. Arrhythmia Electrophysiol.* **7,** 511–517 (2014).
69. Butters, T. D. *et al.* Mechanistic links between Na^+ channel (*SCN5A*) mutations and impaired cardiac pacemaking in sick sinus syndrome. *Circ. Res.* **107,** 126–137 (2010).
70. Makita, N. *et al.* Congenital atrial standstill associated with coinheritance of a novel *SCN5A* mutation and connexin 40 polymorphisms. *Hear. Rhythm* **2,** 1128–1134 (2005).
71. Lei, M. *et al.* Sinus node dysfunction following targeted disruption of the murine cardiac sodium channel gene *Scn5a*. *J. Physiol.* **567,** 387–400 (2005).
72. Zoni-Berisso, M., Lercari, F., Carazza, T. & Domenicucci, S. Epidemiology of atrial fibrillation: European perspective. *Clin. Epidemiol.* **6,** 213–20 (2014).
73. Napolitano, C. The Contradictory Genetics of Atrial Fibrillation: The Growing Gap between Knowledge and Clinical Implications. *J. Cardiovasc. Electrophysiol.* 1–3 (2013). doi:10.1111/jce.12091
74. Ellinor, P. T. *et al.* Cardiac sodium channel mutation in atrial fibrillation. *Hear. Rhythm* **5,** 99–105 (2008).
75. Darbar, D. *et al.* Cardiac sodium channel (*SCN5A*) variants associated with atrial fibrillation. *Circulation* **117,** 1927–1935 (2008).
76. Laitinen-Forsblom, P. J. *et al. SCN5A* mutation associated with cardiac conduction defect and atrial arrhythmias. *J. Cardiovasc. Electrophysiol.* **17,** 480–485 (2006).
77. Rossenbacker, T. *et al.* Novel pore mutation in *SCN5A* manifests as a spectrum of phenotypes ranging from atrial flutter, conduction disease, and Brugada syndrome to sudden cardiac death. *Hear. Rhythm* **1,** 610–615 (2004).
78. Smith, J. G. *et al.* Genome-wide association study of electrocardiographic conduction measures in an isolated founder population: Kosrae. *Hear. Rhythm* **6,** 634–641 (2009).
79. Nikulina, S. Y. *et al.* An Investigation of the Association of the H558R Polymorphism of the *SCN5A* Gene with Idiopathic Cardiac Conduction Disorders. *Genet. Test. Mol. Biomarkers* **19,** 150414120233001 (2015).
80. Mizusawa, Y., Horie, M. & Wilde, A. A. Genetic and Clinical Advances in Congenital Long QT Syndrome. *Circ. J.* **78,** 2827–2833 (2014).
81. Clancy, C. E., Tateyama, M., Liu, H., Wehrens, X. H. T. & Kass, R. S. Non-equilibrium gating in cardiac Na^+ channels: An original mechanism of arrhythmia. *Circulation* **107,** 2233–2237 (2003).
82. Nair, K. *et al.* Escape capture bigeminy: Phenotypic marker of cardiac sodium channel voltage sensor mutation R222Q. *Hear. Rhythm* **9,** 1681–1688 (2012).

83. Bezzina, C. R. *et al.* Compound heterozygosity for mutations (W156X and R225W) in *SCN5A* associated with severe cardiac conduction disturbances and degenerative changes in the conduction system. *Circ. Res.* **92,** 159–168 (2003).
84. Nguyen, T. P., Wang, D. W., Rhodes, T. H. & George, A. L. Divergent biophysical defects caused by mutant sodium channels in dilated cardiomyopathy with arrhythmia. *Circ. Res.* **102,** 364–371 (2008).
85. Alves, L. E., Buser, J. W. & Rose, E. P. Cardiomyopathy due to chronic tachycardias. *JAMA* **253,** 3092 (1985).
86. Luchsinger, J. a. & Steinberg, J. S. Resolution of cardiomyopathy after ablation of atrial flutter. *J. Am. Coll. Cardiol.* **32,** 205–210 (1998).
87. de Groot, S. H. *et al.* Contractile adaptations preserving cardiac output predispose the hypertrophied canine heart to delayed afterdepolarization-dependent ventricular arrhythmias. *Circulation* **102,** 2145–2151 (2000).
88. Moreau, A., Gosselin-Badaroudine, P., Delemotte, L., Klein, M. L. & Chahine, M. Gating pore currents are defects in common with two $Na_V1.5$ mutations in patients with mixed arrhythmias and dilated cardiomyopathy. *J. Gen. Physiol.* **145,** 93–106 (2015).
89. Gosselin-Badaroudine, P. *et al.* A proton leak current through the cardiac sodium channel is linked to mixed arrhythmia and the dilated cardiomyopathy phenotype. *PLoS One* **7,** (2012).
90. Delmar, M. The intercalated disk as a single functional unit. *Hear. Rhythm* **1,** 12–13 (2004).
91. Viswanathan, P. C., Benson, D. W. & Balser, J. R. A common *SCN5A* polymorphism modulates the biophysical effects of an *SCN5A* mutation. *J. Clin. Invest.* **111,** 341–346 (2003).
92. Gui, J., Wang, T., Trump, D., Zimmer, T. & Lei, M. Mutation-specific effects of polymorphism H558R in *SCN5A*-related sick sinus syndrome. *J. Cardiovasc. Electrophysiol.* **21,** 564–73 (2010).
93. Splawski, I. *et al.* Variant of *SCN5A* sodium channel implicated in risk of cardiac arrhythmia. *Science* **297,** 1333–6 (2002).
94. Wang, Q. *et al.* The common *SCN5A* mutation R1193Q causes LQTS-type electrophysiological alterations of the cardiac sodium channel. *J. Med. Genet.* **41,** e66 (2004).
95. NHLBI GO Exome Sequencing Project (ESP). Exome Variant Server. http://evs.gs.washington.edu/EVS/
96. Exome Aggregation Consortium. *Cambridge, MA ExAC* http://exac.broadinstitute.org (2015).
97. Lodder, E. M. & Wilde, A. A. M. Clinical assessment of the pathogenicity of unknown variants in long-QT syndrome: does the pendulum swing back? *J. Cardiovasc. Electrophysiol.* **23,** 643–4 (2012).
98. Kapa, S. *et al.* Genetic testing for long-QT syndrome: distinguishing pathogenic mutations from benign variants. *Circulation* **120,** 1752–1760 (2009).
99. Kapplinger, J. D. *et al.* Enhanced Classification of Brugada Syndrome- and Long QT Syndrome-Associated Genetic Variants in the *SCN5A*-Encoded $Na_V1.5$ Cardiac Sodium Channel. *Circ. Cardiovasc. Genet.* (2015). doi:10.1161/CIRCGENETICS.114.000831
100. Lodder, E. M. & Bezzina, C. R. Genomics of cardiac electrical function. *Brief. Funct. Genomics* (2013). doi:10.1093/bfgp/elt029
101. Albert, C. M. *et al.* Cardiac sodium channel gene variants and sudden cardiac death in women. *Circulation* **117,** 16–23 (2008).
102. Verkerk, A. O. *et al.* Functional $Na_V1.8$ channels in intracardiac neurons: the link between *SCN10A* and cardiac electrophysiology. *Circ. Res.* **111,** 333–43 (2012).
103. Chambers, J. C. *et al.* Genetic variation in *SCN10A* influences cardiac conduction. *Nat. Genet.* **42,** 149–52 (2010).

104. Van Den Boogaard, M. *et al.* A common genetic variant within *SCN10A* modulates cardiac *SCN5A* expression. *J. Clin. Invest.* **124,** 1844–1852 (2014).
105. Milne, J. R. *et al.* Class 1 antiarrhythmic drugs--characteristic electrocardiographic differences when assessed by atrial and ventricular pacing. *Eur. Heart J.* **5,** 99–107 (1984).
106. Balser, J. R. The cardiac sodium channel: gating function and molecular pharmacology. *J. Mol. Cell. Cardiol.* **33,** 599–613 (2001).
107. Courtney, K. R. Slow sodium channel inactivation and the modulated receptor hypothesis Application to phenobarbital. *Biochim. Biophys. Acta - Biomembr.* **642,** 433–437 (1981).
108. Huang, H., Priori, S. G., Napolitano, C., O'Leary, M. E. & Chahine, M. Y1767C, a novel *SCN5A* mutation, induces a persistent Na^+ current and potentiates ranolazine inhibition of $Na_V1.5$ channels. *Am. J. Physiol. Heart Circ. Physiol.* **300,** H288–99 (2011).
109. Moss, A. J. *et al.* Ranolazine shortens repolarization in patients with sustained inward sodium current due to type-3 long-QT syndrome. *J. Cardiovasc. Electrophysiol.* **19,** 1289–93 (2008).
110. Murdock, D. K., Kaliebe, J. & Larrain, G. The use of ranolazine to facilitate electrical cardioversion in cardioversion-resistant patients: a case series. *Pacing Clin. Electrophysiol.* **35,** 302–7 (2012).
111. De Ferrari, G. M. *et al.* Ranolazine in the treatment of atrial fibrillation: Results of the dose-ranging RAFFAELLO (Ranolazine in Atrial Fibrillation Following An ELectricaL CardiOversion) study. *Heart Rhythm* **12,** 872–8 (2015).
112. Gupta, T., Khera, S., Kolte, D., Aronow, W. S. & Iwai, S. Antiarrhythmic properties of ranolazine: A review of the current evidence. *Int. J. Cardiol.* **187,** 66–74 (2015).
113. Zaza, A., Belardinelli, L. & Shryock, J. C. Pathophysiology and pharmacology of the cardiac 'late sodium current'. *Pharmacol. Ther.* **119,** 326–39 (2008).
114. Remme, C. A. & Wilde, A. A. M. Targeting sodium channels in cardiac arrhythmia. *Curr. Opin. Pharmacol.* **15,** 53–60 (2014).
115. Brisson, L. *et al.* Na(V)1.5 enhances breast cancer cell invasiveness by increasing NHE1-dependent H^+ efflux in caveolae. *Oncogene* **30,** 2070–2076 (2011).
116. Pittman, A. M. *et al.* Allelic variation at the 8q23.3 colorectal cancer risk locus functions as a cis-acting regulator of EIF3H. *PLoS Genet.* **6,** (2010).
117. Xing, D. *et al.* Expression of neonatal $Na_V1.5$ in human brain astrocytoma and its effect on proliferation, invasion and apoptosis of astrocytoma cells. *Oncol. Rep.* **31,** 2692–700 (2014).
118. Baptista-Hon, D. T. *et al.* Potent inhibition by ropivacaine of metastatic colon cancer SW620 cell invasion and $Na_V1.5$ channel function. *Br. J. Anaesth.* **113 Suppl ,** i39–i48 (2014).
119. Besson, P. *et al.* How do voltage-gated sodium channels enhance migration and invasiveness in cancer cells? *Biochim. Biophys. Acta - Biomembr.* (2015). doi:10.1016/j.bbamem.2015.04.013
120. Crotti, L. *et al.* Spectrum and prevalence of mutations involving BrS1- Through BrS12-susceptibility genes in a cohort of unrelated patients referred for brugada syndrome genetic testing: Implications for genetic testing. *J. Am. Coll. Cardiol.* **60,** 1410–1418 (2012).
121. Tester, D. J., Will, M. L., Haglund, C. M. & Ackerman, M. J. Compendium of cardiac channel mutations in 541 consecutive unrelated patients referred for long QT syndrome genetic testing. *Hear. Rhythm* **2,** 507–517 (2005).
122. Akai, J. *et al.* A novel SCN5A mutation associated with idiopathic ventricular fibrillation without typical ECG findings of Brugada syndrome. *FEBS Lett.* **479,** 29–34 (2000).
123. Schwartz, P. J. *et al.* A molecular link between the sudden infant death syndrome and the long-QT syndrome. *The New England journal of medicine* **343,** 262–267 (2000).
124. Ackerman, M. J. *et al.* Postmortem molecular analysis of *SCN5A* defects in sudden infant death syndrome. *JAMA* **286,** 2264–2269 (2001).

125. Plant, L. D. *et al.* A common cardiac sodium channel variant associated with sudden infant death in African Americans, *SCN5A* S1103Y. *J. Clin. Invest.* **116,** 430–435 (2006).
126. Watanabe, H. *et al. SCN5A* mutation associated with ventricular fibrillation, early repolarization, and concealed myocardial abnormalities. *Int. J. Cardiol.* **165,** e21–3 (2013).
127. Watanabe, H. *et al.* Electrocardiographic characteristics and *SCN5A* mutations in idiopathic ventricular fibrillation associated with early repolarization. *Circ. Arrhythm. Electrophysiol.* **4,** 874–81 (2011).
128. Ritchie, M. D. *et al.* Genome- and Phenome-Wide Analysis of Cardiac Conduction Identifies Markers of Arrhythmia Risk. *Circulation* **127,** 1377–85 (2013).
129. Jeff, J. M. *et al.* Generalization of Variants Identified by Genome-Wide Association Studies for Electrocardiographic Traits in African Americans. *Ann. Hum. Genet.* **77,** 321–32 (2013).
130. Smith, J. G. *et al.* Genome-wide association studies of the PR interval in African Americans. *PLoS Genet* **7,** e1001304 (2011).
131. Holm, H. *et al.* Several common variants modulate heart rate, PR interval and QRS duration. *Nat. Genet.* **42,** 117–22 (2010).
132. Noseworthy, P. a *et al.* Common genetic variants, QT interval, and sudden cardiac death in a Finnish population-based study. *Circ. Cardiovasc. Genet.* **4,** 305–11 (2011).
133. Sotoodehnia, N. *et al.* Common variants in 22 loci are associated with QRS duration and cardiac ventricular conduction. *Nat. Genet.* **42,** 1068–1076 (2010).
134. Gaunt, T. R. *et al.* Integration of genetics into a systems model of electrocardiographic traits using HumanCVD BeadChip. *Circ. Cardiovasc. Genet.* **5,** 630–8 (2012).
135. Jeff, J. M. *et al. SCN5A* variation is associated with electrocardiographic traits in the Jackson Heart Study. *Circ. Cardiovasc. Genet.* **4,** 139–44 (2011).