Inherited arrhythmia syndromes
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Chapter 1

The cardiac sodium channel gene SCN5A and its gene product Nav1.5: role in physiology and pathophysiology

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Chapter 1

ABSTRACT

The gene SCN5A encodes the main cardiac sodium channel Na\textsubscript{v}1.5. This channel predominates the cardiac sodium current, \(I_{\text{Na}}\), 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\textsubscript{v}1.5.
INTRODUCTION

Voltage-gated sodium channels are responsible for the inward sodium current ($I_{\text{Na}}$) in excitable cells. As such, they induce a fast depolarization, thereby initiating an action potential\(^1\). In the heart, $I_{\text{Na}}$ is crucial for fast impulse propagation through the tissue\(^5\). The main protein generating the cardiac sodium current is the pore-forming alpha subunit Na\(_v\)1.5, encoded by the gene SCN5A\(^3\). 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\(_v\)1.5 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\(_v\)1.5, (ENSG00000183873, HGNC:10593, NCBI Gene ID: 6331), is part of a family of 10 genes encoding sodium channel alpha subunits. Of these Na\(_v\)1.1, Na\(_v\)1.2, Na\(_v\)1.3, Na\(_v\)1.6 and Na\(_v\)2.1 (Na\(_v\)) are the main sodium channels in the central nervous system; Na\(_v\)1.7, Na\(_v\)1.8 and Na\(_v\)1.9 in the peripheral nervous system; Na\(_v\)1.4 in skeletal muscle and Na\(_v\)1.5 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 100 kb 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. 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. Within the sinoatrial node, the central part is devoid of Na\textsubscript{v}1.5 expression, while expression has been demonstrated in the periphery. 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.

**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.
There are four major different SCN5A isoforms, of which SCN5A-003 (NM_000335) is the most abundant transcript in murine and human heart\textsuperscript{7,13}. This transcript, also referred to as the “adult” isoform, replaces the “neonatal” SCN5A-001 (NM_001099404, Na\textsubscript{v}1.5e) 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\textsuperscript{7,13}. SCN5A-001 is also abundantly expressed in neonatal murine brain and at low levels in the adult brain\textsuperscript{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\textsuperscript{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)\textsuperscript{7}. The transcript including 1077Q encodes channels that exhibit less $I_{Na}$\textsuperscript{15}. Remarkably, certain polymorphisms demonstrate more pronounced effects in either the SCN5A-014 or the SCN5A-003 splice variant\textsuperscript{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\textsuperscript{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\textsuperscript{17,19}. The original identified promoter consists of 2.8 kilobases and exceeds exon 1 and partially intron 1\textsuperscript{19}. Several transcription factors that influence gene expression have been identified to date, including Forkhead Box O1 (Foxo1)\textsuperscript{20,21}, nuclear factor-κ B (NF-KappaB)\textsuperscript{22} and TBX5\textsuperscript{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\textsuperscript{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\textsuperscript{25}. 

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**STRUCTURE AND FUNCTION OF NaV1.5**

**Protein structure**

NaV1.5 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, NaV1.5 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, NaV1.5-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 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, \( \text{Na}_\text{V}1.5 \) channels undergo more conformational changes, reaching different states of inactivation, i.e. the ‘intermediate-’ and ‘slow-inactivation’ state. During the cardiac action potential, \( \text{Na}^+ \)-channels never reach the full slow-inactivated state, as this happens only after a time frame of > 60 s\(^2\). The intermediate-inactivated can be reached during the action potential, albeit only by a small fraction of channels\(^3\). 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\(^3\). 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 \( I_{\text{Na,L}} \)\(^{33,34} \). 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” (\( \text{Figure 3A and B} \))\(^{35} \). 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 \( \text{Na}_\text{V}1.5 \) have been identified (extensively reviewed in Abriel, 2010). \( \text{Na}_\text{V}1.5 \) 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 \( \text{SCN1B} \) to \( \text{SCN4B} \) 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 \( \text{Na}_\text{V}1.5 \) a role has been assigned for each one of them. Although results on the direct exact physiological effects of beta-subunits on \( \text{Na}_\text{V}1.5 \)-driven \( \text{Na}^+ \)-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 \( \text{Na}_\text{V}1.5 \) 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 \( \text{SCN1B} \) to \( \text{SCN4B} \) are implicated in different cardiac arrhythmia syndromes\(^37–40 \). For example, \( \text{SCN1B} \), which encodes the \( \beta1 \)-subunit, has been associ-
ated with Brugada syndrome\textsuperscript{38} conduction disease and atrial fibrillation. Apart from the beta-subunits, other proteins interacting and modulating function of Na\textsubscript{V}1.5 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\textsuperscript{41,42}. These interactions as well as information regarding posttranslational modifications of the cardiac sodium channel have been reviewed in detail\textsuperscript{46}.

Figure 3. Na\textsubscript{V}1.5-driven I\textsubscript{Na} in normal conditions. A. The ventricular action potential as a function of time and B. the corresponding I\textsubscript{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\textsubscript{Na} as a consequence of loss-of-function mutation in SCN5A. A. Decreased peak I\textsubscript{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\textsubscript{Na}. Apart from mutations 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 Naᵥ1.5 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. 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, IₐNa,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. To date, loss-of-function mutations have been associated with Brugada syndrome (BrS), progressive cardiac conduction disease (Lev-Lenègre disease), dilated cardiomyopathy (DCM), sick sinus syndrome (SSS) and atrial fibrillation (AF). Mutations resulting in a gain-of-function are causal for Long QT syndrome (LQTS) type 3 and are also more recently implicated in Multifocal ectopic Purkinje related premature contractions (MEPPC). Some gain-of-function mutations are also associated with AF and DCM.

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. Also, in many cases BrS patient present with cardiac conduction slowing. The exact mechanism underlying the ECG abnormalities and the arrhythmia are still controversial. 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. However, apart from incomplete penetrance of mutations, presence of the disease in absence of the familial SCN5A mutation is not uncommon, indicating the genetic complexity of the disease.
**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\textsubscript{v}1.5 in the specialized cardiac conduction system\textsuperscript{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\textsuperscript{52}, after which more reports followed\textsuperscript{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\textsuperscript{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 \textit{SCN5A} \cite{31,58-60,70}. Since expression of \textit{SCN5A} is low in central sinus nodal cells and sodium channels are mostly inactivated during the relatively positive diastolic potential, mutations in \textit{SCN5A} have only small impact on individual primary pacemaker cells. However, in the periphery of the sinus node, in which \textit{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 \cite{68,69}. The role of Na\textsubscript{v}1.5 in sinus node cells has been highlighted in mouse models, demonstrating sinus node dysfunction in Scn5a knockout mice \cite{71} and slowed sinus node conduction upon Na\textsubscript{v}1.5 blockade \cite{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 \cite{69}.

\textit{Atrial fibrillation}

\textit{Atrial fibrillation (AF)} is the most common arrhythmia syndrome in the Western World \cite{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 \cite{73}. Emerging evidence suggests a possible link between mutations in \textit{SCN5A} and familial AF: (i) a high prevalence of \textit{SCN5A} mutations that co-segregated among family members was noted in AF patients \cite{74,75}; (ii) there is a high degree of overlap between atrial fibrillation and other diseases associated with \textit{SCN5A} mutations, i.e. BrS, LQT3 and conduction disease \cite{75-77}; (iii) the common polymorphism Na\textsubscript{v}1.5-H558R, that results in a slight reduction of function, is more prevalent among AF patients than in controls \cite{78,79}. As both gain- and loss-of-function mutations have been described, the possible underlying pathogenic mechanism remains unclear.

\textit{Long QT syndrome type III}

The first mutation described for \textit{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 \cite{61}. From all LQTS patients, 5–10\% carry a mutation in \textit{SCN5A}, whereas 90\% of the patients possess a mutation in the potassium
channel encoding genes \textit{KCNQ1} and \textit{KCNH2} (manifesting as LQT1 and LQT2, respectively)\textsuperscript{80}. Gain-of-function mutations in \textit{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\textsuperscript{81}.

\textit{Multifocal Ectopic Purkinje-Related Premature Contractions}

Recently, the mutation R222Q in \textit{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 \textsuperscript{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 \textit{SCN5A} to a family with patients experiencing a high frequency of exercise-induced premature beats\textsuperscript{63}. Also this variant (I141V) exhibited an increased window current, similar to the R222Q mutation.

\textit{Dilated cardiomyopathy (DCM)}

The association of \textit{SCN5A} mutations and dilated cardiomyopathy (DCM)\textsuperscript{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 \textit{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\textsuperscript{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 straightforward. 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\textsuperscript{95} and ExAC\textsuperscript{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\textsuperscript{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\textsuperscript{99}.

### Table 1. SCN5A genetic variation in the general population

<table>
<thead>
<tr>
<th>Type of mutation</th>
<th>frequency in EVS (n&gt; 6500)</th>
<th>frequency in ExAC (n&gt; 120,000)</th>
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<td>0.01%</td>
</tr>
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<td>0.01%</td>
</tr>
<tr>
<td>frameshift</td>
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<tr>
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<td>0.02%</td>
</tr>
<tr>
<td>Total</td>
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</tr>
</tbody>
</table>

**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\textsuperscript{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 \textit{SCN5A} and \textit{SCN10A} genomic region on chromosome 3 to be associated with variation in QTc-interval, QRS duration and PR-interval\textsuperscript{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 \textit{SCN5A} locus has been implicated in the risk of sudden cardiac death in a candidate gene study in the general population\textsuperscript{101}. Whether the effects of the independent GWAS signals in \textit{SCN5A} and \textit{SCN10A} (the genes are juxtaposed at 60 kb from each other at chr3p22) are mediated through \textit{SCN5A} alone\textsuperscript{24} or partially to the Na\textsubscript{v}1.8 encoding gene \textit{SCN10A} as well\textsuperscript{102,103} is still a matter of debate. Of note, transcript levels of \textit{SCN10A} did not exceed the detection limit of RNA sequencing in ventricular tissues\textsuperscript{104}, suggesting a limited role of this gene in cardiomyocytes.

\textbf{NA\textsubscript{v}1.5 AS A PHARMACOLOGICAL TARGET}

The cardiac sodium channel Na\textsubscript{v}1.5 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\textsuperscript{105}. The mode of action of these blockers may depend on the biophysical state of the Na\textsubscript{v}+-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”\textsuperscript{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\textsuperscript{+} and Ca\textsuperscript{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\textsuperscript{106}.

Apart from acting on peak sodium current, sodium channel blockers also impact on the late component of the sodium current (I\textsubscript{Na,L}) to different extents. Drugs that inhibit mainly I\textsubscript{Na,L} are of potential clinical interest as an increase in I\textsubscript{Na,L} is involved in different conditions. Ranolazine, the most selective I\textsubscript{Na,L} that is currently in clinical use, is approved by the FDA for the treatment of angina pectoris. During ischemia, in which I\textsubscript{Na,L} is en-
hanced, ranolazine reduces intracellular Ca\textsuperscript{2+} concentration indirectly through the Na\textsuperscript{+}/Ca\textsuperscript{2+}-exchanger, and thereby cardiac workload. As an antiarrhythmic agent, ranolazine is especially of interest in the treatment of LQT3, where $I_{\text{Na,L}}$ directly prolongs action potential duration and causes QT prolongation, as proven by several preclinical and clinical studies\textsuperscript{108,109}. Furthermore, different studies suggest that ranolazine is a potential candidate in treating atrial fibrillation\textsuperscript{110-112}. Given that ranolazine can exert unwanted effects by blocking repolarizing potassium currents\textsuperscript{113}, possibly evoking drug-induced Long QT-syndrome, more selective drugs are currently under development\textsuperscript{114}.

**NON-CANONICAL ROLES OF Na\textsubscript{v}1.5**

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\textsubscript{v}1.5 of which the “neonatal” isoform is expressed and functional in among others breast cancer cells, colon cancer and brain astrocytoma\textsuperscript{115-118}. In these cells, Na\textsubscript{v}1.5 function is associated with increased invasiveness and the development of metastases\textsuperscript{119}. In breast cancer cells, Na\textsubscript{v}1.5 co-localizes with the Na\textsuperscript{+}/H\textsuperscript{+}-exchanger (NHE-1)\textsuperscript{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\textsuperscript{+}-ions is also of importance, as blocking the channels in cancer cells directly with sodium current blockers affects the degree of invasion\textsuperscript{117,118}. Recent studies have identified Na\textsubscript{v}1.5 in endosomes of macrophages present within lesions of the neurological disease multiple sclerosis\textsuperscript{10}. Here the channels contribute to phagocytosis and pH-regulation within the endosome. It is suggested that also for this disease, targeting Na\textsubscript{v}1.5 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


