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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(Weidmann 1955). In the heart, I_{Na} is crucial for fast impulse propagation through the tissue(Buchanan et al. 1985). The main protein generating the cardiac sodium current is the pore-forming alpha subunit $Na_V1.5$, encoded by the gene *SCN5A* (Gellens et al. 1992; Abriel 2010). Since the cloning of this gene in 1992(Gellens et al. 1992), 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 (Remme, 2013). We here provide an overview of the current knowledge concerning the regulation and function of the gene *SCN5A* and its corresponding protein

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The corresponding Gene Wiki entry for this review can be found here: http://en.wikipedia.org/wiki/Nav1.5 “

$Na_V1.5$ 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(Abriel 2010), posttranslational modifications(Marionneau & Abriel 2015)), human genetics and splice regulation (Schroeter et al. 2010). 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 Catterall, 2014). *SCN5A* is a large, highly conserved gene that is present from platypus to birds and human (Catterall 2014). 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(Holm et al. 2002) and in macrophages(Black et al. 2013). Also, the “neonatal” splice isoform of *SCN5A* (see below) is expressed in the central nervous system and in certain types of cancer (Catterall 2014). 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 (Remme et al. 2009). Within the sinoatrial node, the central part is devoid of $Na_v1.5$ expression, while expression has been demonstrated in the periphery(Lei et al. 2004). Accross the ventricular wall, a transmural expression gradient exists, as reflected by higher expression of *SCN5A* in the subendocardial layer as compared to the subepicardium(Remme et al. 2009).

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. In Table 1 we provide an overview of the annotated splice variants with the corresponding reference IDs in different databases and papers. A clear comprehensive overview of the functional differences between the different splice isoforms is given in a recent review (Schroeter et al. 2010).

There are four major different coding *SCN5A* isoforms, of which *SCN5A*-003 (NM_000335) is the most abundant transcript in murine and human heart (Onkal et al. 2008; Schroeter et al. 2010) (figure 1). 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. (Onkal et al. 2008; Schroeter et al. 2010). *SCN5A*-001 is also abundantly expressed in neonatal murine brain and at low levels in adult brain (Chioni et al. 2005). Functionally these two isoforms differ significantly: compared to the adult isoform, the neonatal splice isoform exhibits slower rate of activation and inactivation and a depolarized shift in voltage dependence of activation(Onkal et al. 2008). 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, howeverthis splice isoform has not been found in human tissue as yet (Schroeter et al. 2010). *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 human heart albeit less abundantly than *SCN5A*-003 (ratio approximately 1:2) (Makielski et al. 2003; Schroeter et al. 2010). The transcript including 1077Q encodes channels that exhibit less I_{Na}(Makielski et al. 2003). Remarkably, certain polymorphisms demonstrate more pronounced effects in either the *SCN5A-014* or the *SCN5A*-003 splice variant. (Tan et al. 2005). 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 transcriptional regulation.

Interestingly, the different exons 1 are partially species specific as they are different between the murine and human genome (van Stuijvenberg et al. 2010; Shang & Dudley 2005). 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 (Yang et al. 2004; van Stuijvenberg et al. 2010). The original identified promoter consists of 2.8 kilobases and exceeds exon1 and partially intron 1 (Yang et al. 2004). Several transcription factors that influence gene expression have been identified to date, including Forkhead Box O1 (Foxo1)(Cai et al. 2014; Mao et al. 2012), nuclear factor-κ B (NF-KappaB) (Shang et al. 2008) and TBX5 (Arnolds et al. 2012; Van Den Boogaard et al. 2012). Foxo1 and NF-KappaB are both involved in gene regulation upon oxidative stress, 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 (Arnolds et al. 2012; Van Den Boogaard et al. 2012). 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 (Daimi et al. 2015).

Structure and function of $Na_V1.5$

Protein structure and function

$Na_V1.5$ 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) (Chen-Izu et al. 2015; Catterall 2014). As 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 1ms. 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 outward, 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 (West et al. 1992; Kellenberger et al. 1996). 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 seconds(O'Reilly et al. 1999). The intermediate-inactivated state however, can be reached during the action potential, albeit only by a small fraction of channels (Nuss et al. 1995; Veldkamp et al. 2003). 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 (Groome 2014).

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 '$I_{Na,L}$' (Sakmann et al. 2000; Maltsev et al. 1998). Finally, some channels may reactivate during the repolarizing phase of the action potential at a range of potentials where inactivation is not complete and shows overlap with activation, generating the so-called "window current"

(Figure 3A and 3B) (Attwell et al. 1979). Both the window current and the sustained current can play important roles in genetic and acquired cardiac diseases, as discussed below.

Sub-units 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 (Meadows & Isom 2005; Abriel 2010), 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 (Meadows & Isom 2005). 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 (Watanabe et al. 2009; Watanabe et al. 2008; Hu et al. 2009; Medeiros-Domingo et al. 2007). For example, *SCN1B*, which encodes the β_1-subunit, has been associated with Brugada syndrome (Watanabe et al. 2008), 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 (Cerrone et al. 2014; Herren et al. 2013) These interactions and postranslation modifications of the cardiac sodium channel have been reviewed in detail (see Abriel, 2010).

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 (Remme et al. 2006). 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 (Lupoglazoff et al. 2002; Neu et al. 2010; Lopez et al. 2011; Frigo et al. 2007; Bezzina et al. 2003; Baskar et al. 2014). 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 the 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 (Nakajima et al. 2013; Bezzina et al. 1999; Remme et al. 2006).

To date, loss-of-function mutations have been associated with Brugada syndrome (BrS) (Chen et al. 1998; Bezzina et al. 1999; Remme et al. 2006) progressive cardiac conduction disease (Lev-Lenègre disease) (Schott et al. 1999; Tan et al. 2001), dilated cardiomyopathy (DCM) (McNair et al. 2004; Olson et al. 2005; Laurent et al. 2012; Bezzina et al. 2003), sick sinus syndrome (Benson et al. 2003; Smits et al. 2005) and atrial fibrillation (Makiyama et al. 2008). Mutations resulting in a gain-of-function are causal for Long QT syndrome type 3 (Wang et al. 1995; Remme et al. 2006) and are also more recently implicated in Multifocal ectopic Purkinje-related premature contractions (MEPPC)(Mann et al. 2012; Swan et al. 2014; Laurent et al. 2012). Some gain-of-function mutations are also associated with AF and DCM (Olson et al. 2005).

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 (Coronel et al. 2005). Also, in many cases BrS patient present with cardiac conduction slowing. The exact mechanism underlying the ECG abnormalities and the arrhythmia are still controversial (Hoogendijk et al. 2011). 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 (Lippi et al. 2012). 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 (Probst et al. 2009; Marsman et al. 2013).

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 (Remme et al. 2009). 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 (Schott et al. 1999), after which more reports followed (Tan et al. 2001).

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 (Abe et al. 2014; Butters et al. 2010; Benson et al. 2003). 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* (Makita et al. 2005; Benson et al. 2003; Smits et al. 2005; Veldkamp et al. 2003; Makiyama et al. 2008). 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(Lei et al. 2004), 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 (Abe et al. 2014; Butters et al. 2010). The role of $Na_v1.5$ in sinus node cells has been highlighted in mouse models, demonstrating sinus node dysfunction in *Scn5a* knockout mice(Lei et al. 2005) and slowed sinus node conduction upon $Na_v1.5$ blockade(Lei et al. 2004). 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 (Butters et al. 2010).

Atrial fibrillation—Atrial fibrillation (AF) is the most common arrhythmia syndrome in the Western World (Zoni-Berisso et al. 2014). 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 (Napolitano 2013). 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 (Ellinor et al. 2008; Darbar et al. 2008); (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 (Laitinen-Forsblom et al. 2006; Rossenbacker et al. 2004; Darbar et al. 2008); (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 (Smith et al. 2009; Nikulina et al. 2015). 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 (LQT type III). Patients with LQT syndrome exhibit prolonged QT-intervals at the ECG, which is reflected as increased action potential duration at the cellular level (Figure 5A). Moreover, patients are at increased risk for the development of polymorphic ventricular tachycardia, specifically torsade de pointes (Wang et al. 1995). From all successfully genotyped LQTS patients, 5–10% carry a mutation in *SCN5A*, whereas 90% of the genotyped patients possess a mutation in the potassium channel encoding genes *KCNQ1* and *KCNH2* (manifesting as LQT1 and LQT2, respectively)(Mizusawa et al. 2014). 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 (Figure 5B). 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 (Figure 5C4B). 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 (Clancy et al. 2003)

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 (Nair et al. 2012; Laurent et al. 2012; Mann et al. 2012). 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 simulated 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 (Swan et al. 2014). 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) (Olson et al. 2005; Laurent et al. 2012; Bezzina et al. 2003; McNair et al. 2004), 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 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 (Nguyen et al. 2008). Several pathophysiological mechanisms have been proposed. First, DCM could develop secondary to frequent arrhythmia or sinus node dysfunction, as is observed with MEPPC. (Alves et al. 1985; Luchsinger & Steinberg 1998). In 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 (de Groot et al. 2000). 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 (Moreau et al. 2015; Gosselin-Badaroudine et al. 2012) 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 (Gosselin-Badaroudine et al. 2012). Finally, the mechanism by which *SCN5A*-mutation cause DCM could be solely non-electrical. At the intercalated disks of cardiomyocytes, $Na_V1.5$ is part of a macromolecular complex (Delmar 2004) 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 non-coding 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* (Yang et al. 2004; Van Den Boogaard et al. 2012). As such, these SNPs affect conduction in healthy and diseased patients (Syrris et al. 2006) and arrhythmia susceptibility in *SCN5A* mutation carriers (Clancy et al. 2003). 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 (Viswanathan et al. 2003; Gui et al. 2010), while presence in a wildtype channel reduces current, depending on the presence of the splice variant that includes Q1077 (Makielski et al. 2003). Other studied polymorphisms include S1102Y (Splawski et al. 2002) (10% in Blacks) and R1193Q (8% in Asians) (Wang et al. 2004), 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 (see Table 2 for details). 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 (Kapplinger et al. 2015). With the advent of exome sequencing, large panels of individuals have now been sequenced for *SCN5A*, the results of which are publicly available through online databases such as the Exome Variant Server (NHLBI GO Exome Sequencing Project (ESP) n.d.) and ExAC (Exome Aggregation Consortium 2015). 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 3). 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 disorders 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 (Lodder & Wilde 2012; Kapa et al. 2009). In contrast, variants that dramatically alter protein structure (splice, stop gain and frameshift) are very rare in these cohorts (Table 3), 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 pathogenic variants from background genetic noise. Unfortunately, even then many variants remain in the limbo as VUS (Kapplinger et al. 2015).

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 Lodder and Bezzina, 2013). 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 (see Table 3 and (Lodder & Bezzina 2013) for a recent overview). 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 (Albert et al. 2008). 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 (Van Den Boogaard et al. 2012) or partially to the $Na_V1.8$ encoding gene *SCN10A* as well (Verkerk et al. 2012; Chambers et al. 2010) is still a matter of debate. Of note, transcript levels of *SCN10A* did not exceed the detection limit of RNA sequencing in ventricular tissues(Van Den Boogaard et al. 2014), 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 class IA, IB and IC, depending on their ability to change the length of the cardiac action potential (Milne et al. 1984). 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” (Balser 2001; Courtney 1981). 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 (Balser 2001).

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, where $I_{Na,L}$ is enhanced, 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 (Huang et al. 2011; Moss et al. 2008). Furthermore, different studies suggest that ranolazine is a potential candidate in treating atrial fibrillation (Murdock et al. 2012; De Ferrari et al. 2015; Gupta et al. 2015). Given that ranolazine can exert unwanted effects by blocking repolarizing potassium currents (Zaza et al. 2008), possibly evoking drug-induced Long QT-syndrome, more selective drugs are currently under development (Remme & Wilde 2014).

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 amongst others breast cancer cells, colon cancer and brain astrocytoma (Brisson et al. 2011; Pittman et al. 2010; Xing et al. 2014; Baptista-Hon et al. 2014). In these cells $Na_V1.5$ function is associated with increased invasiveness and the development of metastases (Besson et al. 2015). In breast cancer cells, $Na_V1.5$ co-localizes with the Na^+/H^+-exhanger (NHE-1) (Brisson et al. 2011), 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(Xing et al. 2014; Baptista-Hon et al. 2014).

Recent studies have identified $Na_V1.5$ in endosomes of macrophages present within lesions of the neurological disease multiple sclerosis (Black et al. 2013). 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.

Summary

SCN5A and its gene product $Na_V1.5$ are essential for proper cardiac function. The $Na_V1.5$ mediated Na^+-current I_{Na} underlies the fast depolarization phase of the cardiac action potential. Mutations in *SCN5A* are implicated in several inherited cardiac arrhythmia syndromes and dilated cardiomyopathy as described in detail above. Genetic variation in *SCN5A* in the general population is associated with variation in ECG-parameters. While a tremendous amount has been learned about the channel and this knowledge has lead to improved clinical care, much remains to be discovered. The underlying mechanisms of some of the *SCN5A*-associated arrhythmia syndromes (i.e. Brugada syndrome) and the genetic variability in the general population remain to be solved. Furthermore, the non-canonical roles of *SCN5A* are interesting new avenues to explore.

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Although we have tried to refer as much as possible to the original research papers, it is impossible to cite all of the more than 1500 papers on *SCN5A* listed in Pubmed today. We therefore apologize to all researchers whose original articles were not directly cited. This review and the corresponding Gene Wiki article are written as part of the Cardiac Gene Wiki Review series--a series resulting from a collaboration between the journal GENE, the Gene Wiki Initiative, and the BD2K initiative. The Cardiac Gene Wiki Initiative is supported by National Institutes of Health (GM089820and GM114833). Additional support for Gene Wiki Reviews is provided by Elsevier, the publisher of GENE. We acknowledge the support from the “Netherlands CardioVascular Research Initiative”: the Dutch Heart Foundation, Dutch Federation of University Medical Centres, the Netherlands Organisation for Health Research and Development and the Royal Netherlands Academy of Sciences (PREDICT project).

Abbreviation list, Veerman et al:

AF	atrial fibrillation
BrS	Brugada syndrome
DCM	dilated cardiomyopathy
ECG	electrocardiogram
Foxo1	Forkhead Box O1
GWAS	Genome Wide Association Study
$\boldsymbol{I_{Na}}$	sodium current
$\boldsymbol{I_{Na,L}}$	late component of the sodium current
Kb	kilobases
LQTS	Long QT syndrome
MEPPC	Multifocal ectopic Purkinje-related premature contractions
miR	microRNA
$\boldsymbol{Na_V1.5}$	voltage-gated sodium channel type v alpha subunit
NF-KappaB	nuclear factor-κ B

NHE-1	Na^+/H^+-exhanger
Ros	reactive oxygen species
SNPs	single nucleotide polymorphisms
Tbx5	T-Box 5
UTR	untranslated region
VUS	variant of unknown significance

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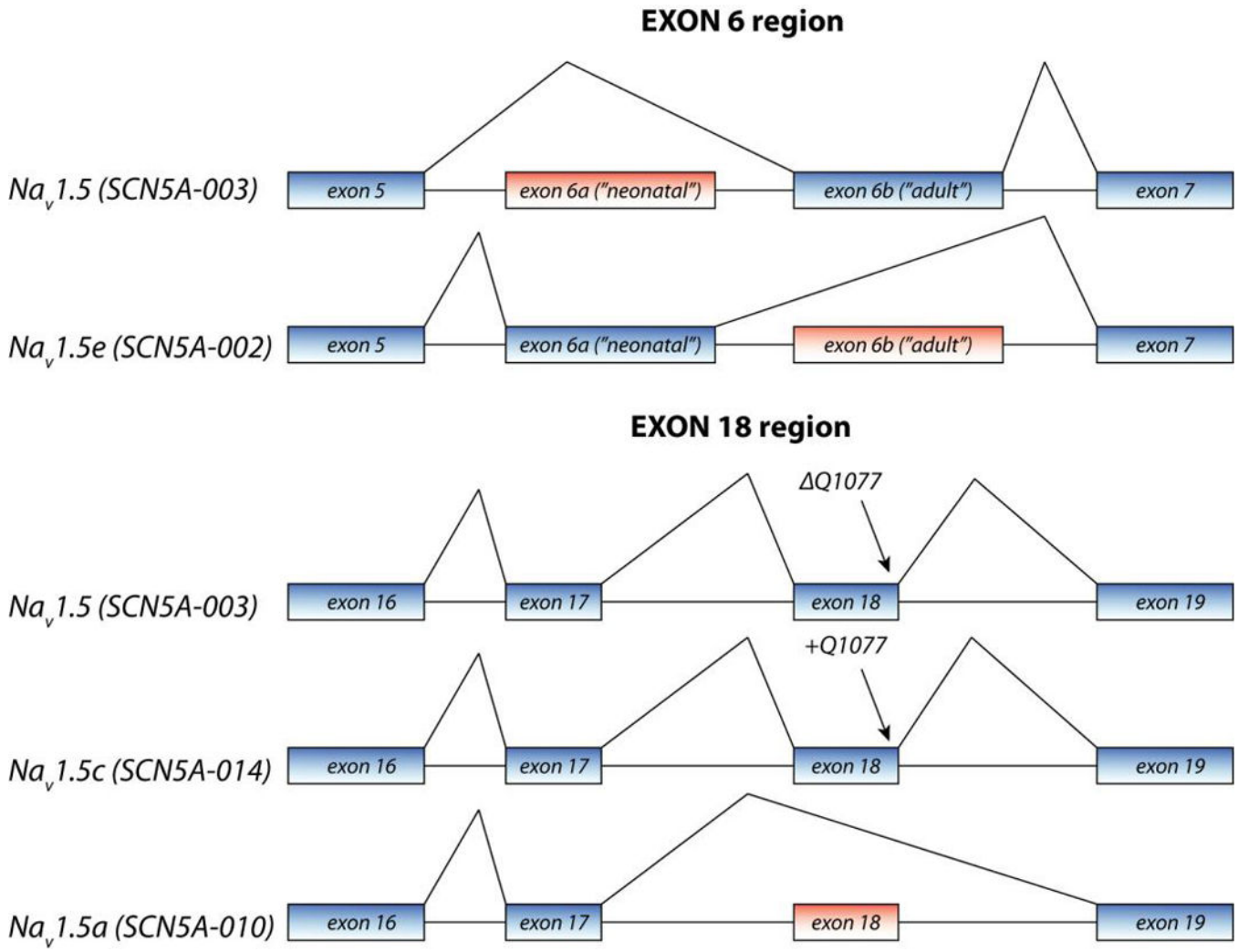

Figure 1: Illustrative scheme of splice variants of *SCN5A*.

Annotations are according to Schroeter et al (2010), with in parenthesis the ID as can be found in the Ensembl database (see Table 1). Alternative splicing mainly occurs at the region of exon 6 and exon 18.

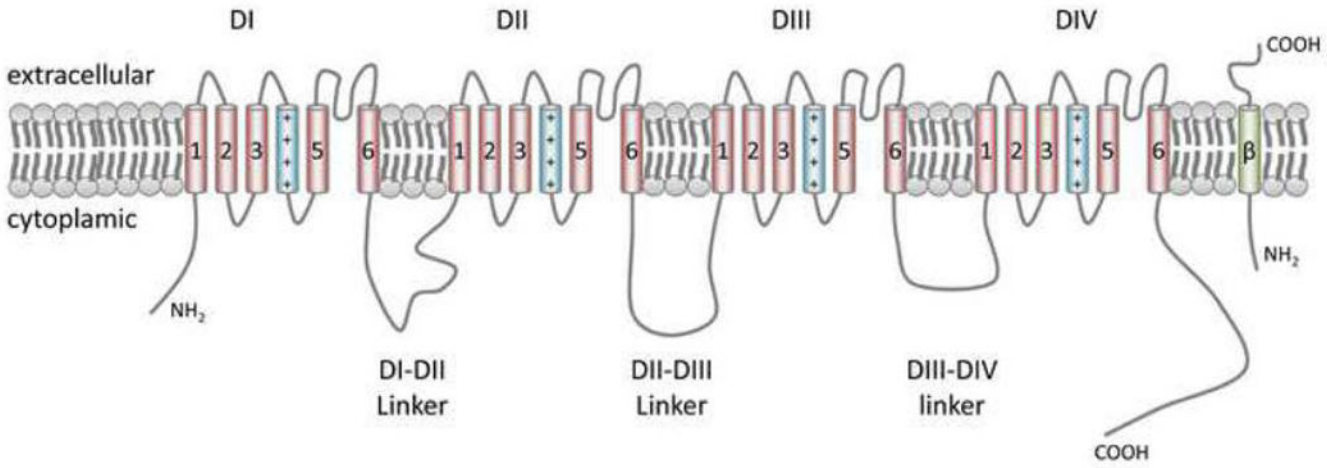

Figure 2. $Na_V1.5$ protein structure.

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.

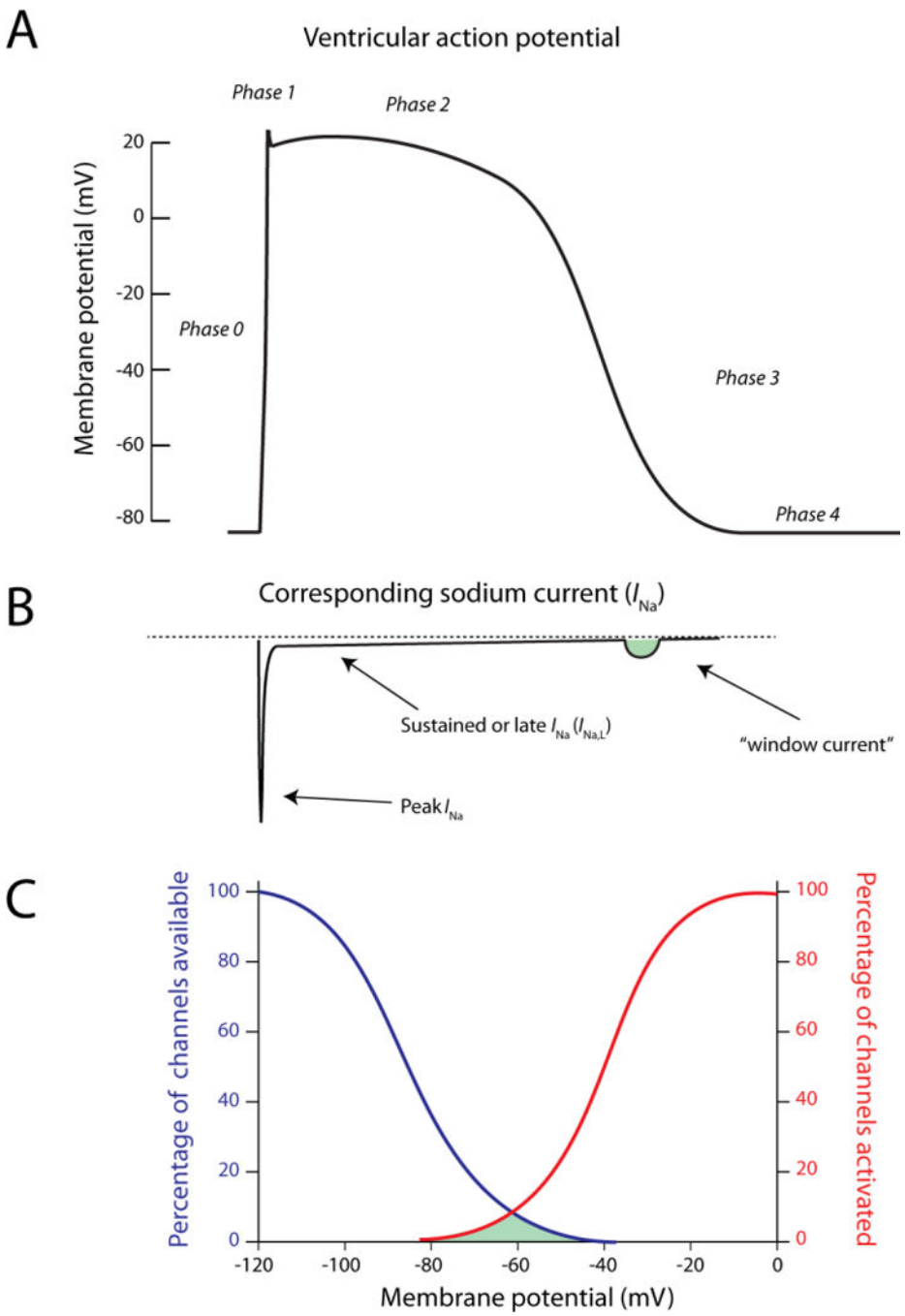

Figure 3. $Na_v1.5$-driven I_{Na} in normal conditions.

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 in which inactivation and activation are overlapping.

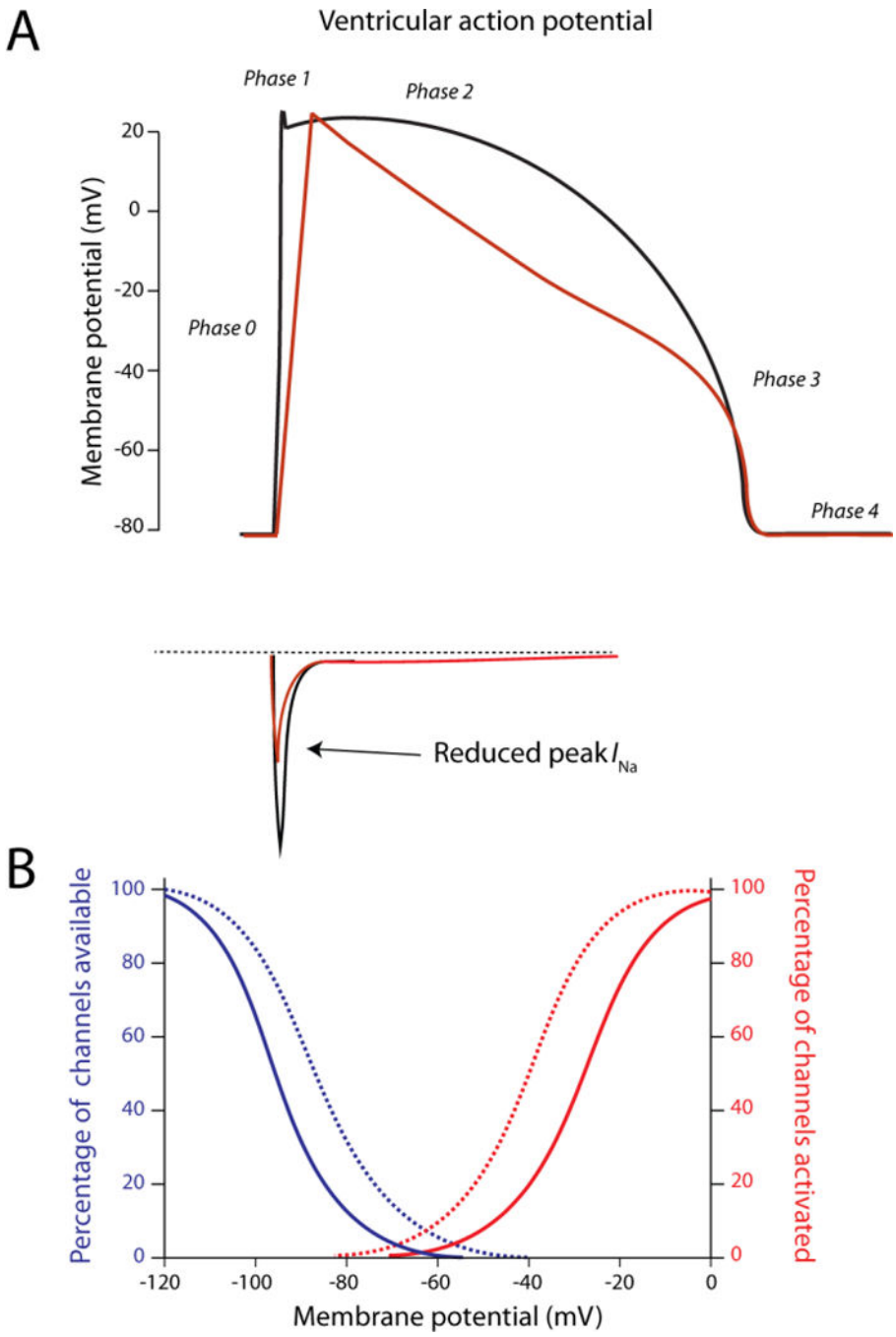

Figure 4. Reduced function of I_{Na} as a consequence of loss-of-function mutation in SCN5A.
A) Decreased peak I_{Na} lowers the upstroke velocity of the action potential (red trace). **B)** Shifts in voltage dependence of (in)activation (dashed lines) that result in loss-of-function of I_{Na}. Apart 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.

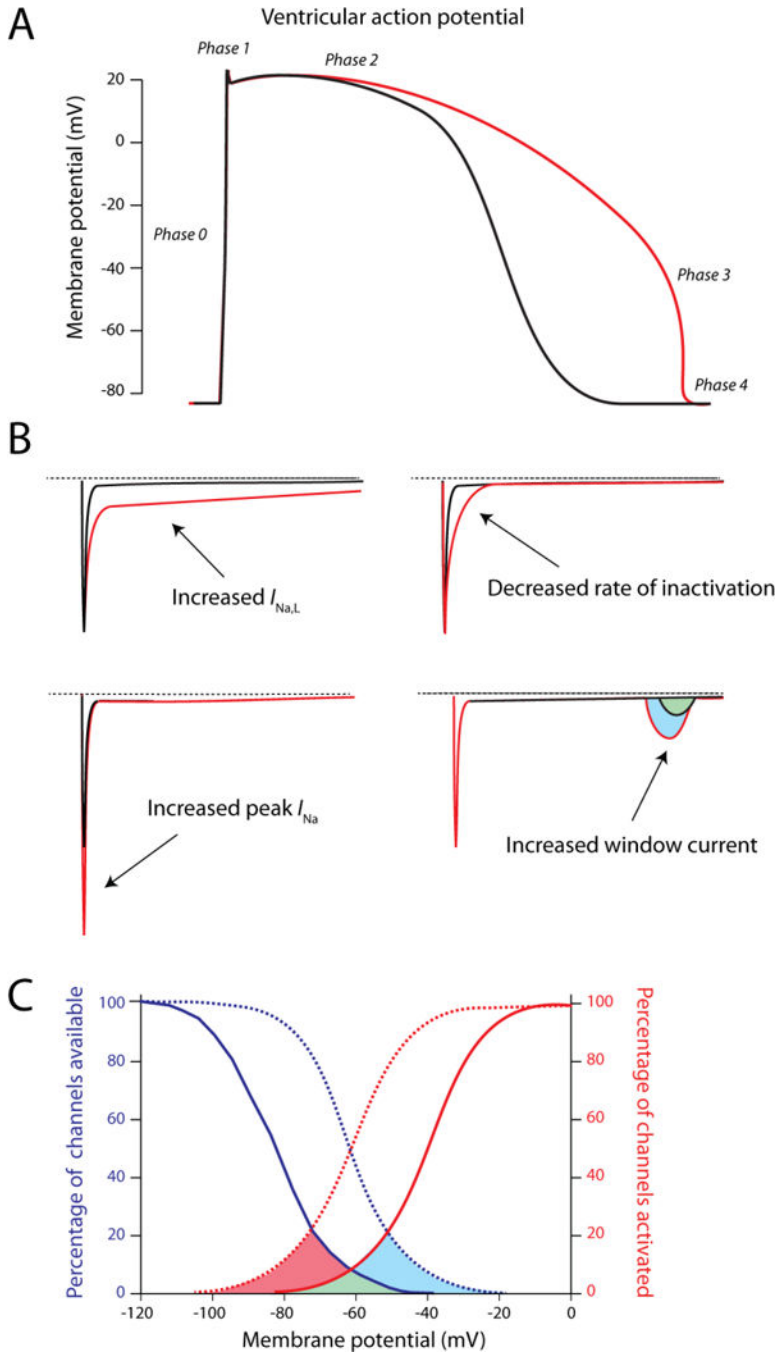

Figure 5: Functional effects of gain-of-function mutations in SCN5A.

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 can be responsible for the 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.

Table 1,

SCN5A transcript variants

Overview of the described splice variants in *SCN5A.* As different sources refer to the different variants with different numbers and letters, we here aim to provide an unambiguous link between the different annotations. Ensembl transcript number and ID refer to identifications of the Ensembl consortium (www.ensembl.org); CCDS refers to the consensus cDNA (http://www.ncbi.nlm.nih.gov/CCDS/CcdsBrowse.cgi) number. Uniprot ID refers to the reference protein ID (http://www.uniprot.org/); RefSeq ID and cDNA name refers to the reference transcript and protein IDs and the isoform names of NCBI respectively (http://www.ncbi.nlm.nih.gov). Characteristics provides a short summary of the essential differences between the isoforms.

Ensembl transcript nr	Ensembl ID	length of cDNA	length of protein	CCDS	UniPROT ID	RefSeq IDs (transcript, protein)	NCBI names (transcript, protein)	cDNA name (Schroeter et al)	characteristics
SCN5A-014	ENST00000333535	8456	2016	CCDS46796	Q14524	NM_198056, NP_932173	transcript variant 1, isoform a	$Na_V1.5c$	“adult” exon 6b, inclusion of Q1077
SCN5A-003	ENST00000423572	8362	2015	CCDS46797	Q14524	NM_000335, NP_000326	transcript variant 2, isoform b	$Na_V1.5$	alternative 5’UTR, ΔQ1077, “adult” exon 6b
SCN5A-001	ENST00000413689	8504	2016	CCDS46799	H9KVD2	NM_001099404, NP_001092874	transcript variant 3, isoform c	$Na_V1.5e$	“neonatal” exon 6a, inclusion of Q1077
SCN5A-002	ENST00000455624	7170	1983	CCDS54570	E9PHB6	NM_001160160, NP_001153632	transcript variant 5, isoform e	$Na_V1.5e$	alternative 5’UTR, ΔQ1077, “neonatal” exon 6a
SCN5A-004	ENST00000414099	8303	1998	CCDS46798	E9PG18	-	-	$Na_V1.5f$	alternative 5’UTR, Q1077, lacking exon 24
SCN5A-008	ENST00000450102	6284	1962	CCDS54569	K4DIA1	-	-	n.d.	“neonatal” exon 6a, lacking exon 18, alternative 3’UTR
SCN5A-010	ENST00000449557	5898	1962	-	A0A0A0MT39	-	-	$Na_V1.5a$	“adult” exon 6b, lacking exon 18, alternative 3’UTR
SCN5A-201	ENST00000425664	8450	1998	CCDS46798	E9PG18	NM_001099405, NP_001092875	transcript variant 4, isoform d	$Na_V1.5f$	predicted transcript, Q1077, lacking exon 24
SCN5A-202	ENST00000451551	8343	1962	CCDS54569	K4DIA1	NM_001160161, NP_001153633	transcript variant 6, isoform f	n.d.	predicted transcript, lacking exon 18, “neonatal” exon 6a
SCN5A-203	ENST00000612060	714	223	-	Q86V90	-	-	n.d.	incomplete
SCN5A-006	ENST00000327956	335	65	-	A3EY21	-	-	n.d.	incomplete
SCN5A-005	ENST00000491944	1382	-	-	-	-	-	n.d.	non-coding
SCN5A-013	ENST00000476683	774	-	-	-	-	-	n.d.	non-coding
SCN5A-011	ENST00000464652	591	-	-	-	-	-	n.d.	non-coding

Table 2,
Diseases and phenotypes associated with SCN5A genetic variation

Overview of the diseases and phenotypes associated with genetic variation in SCN5A, with the level of evidence, Online Mendelian Inheritance in Man (OMIM) database numbers and pertinent references.

OMIM	disease/phenotype	evidence	references
614022	Familial Atrial Fibrillation	Candidate gene approach	(Laitinen-Forsblom et al. 2006; Ellinor et al. 2008; Darbar et al. 2008)
601144	Brugada syndrome	Candidate gene approach, cosegregation, functional studies, confirmed in many patients	(Crotti et al. 2012; Bezzina et al. 1999; Chen et al. 1998; Remme et al. 2006)
601154	Dilated Cardiomyopathy	Linkage analysis, contradicting results	(Olson et al. 2005; Laurent et al. 2012; Bezzina et al. 2003; McNair et al. 2004)
113900	Cardiac Conduction Disease	Linkage analysis and functional studies	(Schott et al. 1999; Tan et al. 2001)
603830	Long QT syndrome	Linkage analysis and functional studies	(Tester et al. 2005; Wang et al. 1995; Bezzina et al. 1999; Veldkamp et al. 2003; Remme et al. 2006)
608567	Sick sinus syndrome	Candidate gene approach	(Benson et al. 2003)
603829	Familial ventricular fibrillation	Candidate gene approach	(Akai et al. 2000)
272120	Sudden infant death syndrome, susceptibility to	Candidate gene approach	(Schwartz et al. 2000; Ackerman et al. 2001; Plant et al. 2006)
613601	Early Repolarisation syndrome	Candidate gene approach	(Watanabe et al. 2013; Watanabe et al. 2011)
108980	QRS interval duration	GWAS signal replicated in many independent studies	(Ritchie et al. 2013; Jeff et al. 2013; Smith et al. 2009)
610141	QTc interval duration	GWAS signal replicated in many independent studies	(Smith et al. 2011; Holm et al. 2010; Noseworthy et al. 2011; Sotoodehnia et al. 2010; Gaunt et al. 2012; Chambers et al. 2010)
108980	PR interval duration	GWAS signal replicated in many independent studies	(Jeff et al. 2011; Smith et al. 2011; Holm et al. 2010; Chambers et al. 2010; Sotoodehnia et al. 2010; Gaunt et al. 2012)
-	Multifocal ectopic Purkinje-related premature contractions (MEPPC)	Candidate gene approach	(Laurent et al. 2012; Mann et al. 2012; Nair et al. 2012; Swan et al. 2014)

Table 3:
SCN5A genetic variation in the general population.

Frequencies of different types of variants with a minor allele frequency of <1% in the general population as can be found in the databases of Exome Variant Server (EVS) (http://evs.gs.washington.edu/EVS/) and Exome Aggregation Consortium (ExAC) (http://exac.broadinstitute.org/)

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%**