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Review

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SCN5A channelopathy: arrhythmia, cardiomyopathy, epilepsy and beyond

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Influx of sodium ions through voltage-gated sodium channels in cardiomyocytes is essential for proper electrical conduction within the heart. Both acquired conditions associated with sodium channel dysfunction (myocardial ischaemia, heart failure) as well as inherited disorders secondary to mutations in the gene *SCN5A* encoding for the cardiac sodium channel Nav1.5 are associated with life-threatening arrhythmias. Research in the last decade has uncovered the complex nature of Nav1.5 distribution, function, in particular within distinct subcellular subdomains of cardiomyocytes. Nav1.5-based channels furthermore display previously unrecognized non-electrogenic actions and may impact on cardiac structural integrity, leading to cardiomyopathy. Moreover, *SCN5A* and Nav1.5 are expressed in cell types other than cardiomyocytes as well as various extracardiac tissues, where their functional role in, e.g. epilepsy, gastrointestinal motility, cancer and the innate immune response is increasingly investigated and recognized. This review provides an overview of these novel insights and how they deepen our mechanistic knowledge on *SCN5A* channelopathies and Nav1.5 (dys)function.

This article is part of the theme issue 'The heartbeat: its molecular basis and physiological mechanisms'.

1. Introduction

Influx of sodium ions through voltage-gated sodium channels in cardiomyocytes initiates the cardiac action potential and is essential for excitability of myocardial cells and proper electrical conduction within the heart. The importance of cardiac sodium channels is underscored by the occurrence of potentially lethal arrhythmias in the setting of acquired conditions associated with sodium channel dysfunction (myocardial ischaemia, heart failure) as well as inherited disorders secondary to mutations in the gene *SCN5A* encoding for the cardiac sodium channel Nav1.5 [1]. While genetic, electrophysiological and molecular studies have provided insight into the (dys)function and (dys)regulation of *SCN5A* and Nav1.5, it has become increasingly clear that sodium channel distribution, function and regulation is more complicated than traditionally assumed, in particular within distinct subcellular subdomains of cardiomyocytes [2]. Nav1.5-based channels furthermore display previously unrecognized non-electrogenic actions and may impact on cardiac structural integrity, thereby also potentially affecting arrhythmogenesis [3]. Moreover, *SCN5A* and Nav1.5 are expressed in cell types other than cardiomyocytes as well as various extracardiac tissues, where their functional role is increasingly investigated and recognized. *SCN5A* mutations have now been associated with (sudden unexpected death in) epilepsy and gastrointestinal disorders, and a role for Nav1.5 in smooth muscle cell (SMC) function, cancer, innate immune response and inflammation has been reported. This review provides an overview of these novel insights and how they deepen our mechanistic knowledge on sodium channel (dys)function and their role in cardiac disorders, ultimately facilitating development of novel strategies for diagnosis, risk stratification and treatment in patients with *SCN5A* channelopathies.

2. Sodium channel structure, distribution and function

(a) Cardiac sodium channel structure and mode of action

The sodium channel family comprises a total of nine genes (*SCN1A-SCN5A, SCN7A-SCN11A*), of which the *SCN5A* gene located on human chromosome 3p22 encodes Nav1.5, the pore-forming alpha subunit of the cardiac sodium channel. Nav1.5 is made up of a cytoplasmic N terminus, four internally homologous domains (DI-DIV; consisting of six transmembrane α-helical segments, S1–S6) interconnected by cytoplasmic linkers, and a cytoplasmic C terminal domain [4]. The DI-DIV domains fold around an ion-conducting pore; during membrane depolarization, outward movement of the positively charged S4 segments (which acts as voltage sensor) results in the opening of the channel pore, allowing for sodium influx [5]. As a result, the membrane is further depolarized (phase 0 of the cardiac action potential), leading to L-type calcium channel activation, calcium influx and contraction. Subsequent fast and slow inactivation closes the channel pore, until membrane repolarization allows the channel to recover from inactivation and once again become available for activation [6,7]. During physiological conditions, activation and inactivation of sodium channels is closely regulated, but alterations in these processes in the setting of acquired and inherited sodium channel dysfunction may set the stage for electrical disturbances and arrhythmias (as discussed in more detail in the next sections).

(b) *SCN5A*/Nav1.5 distribution in heart and subcellular domains of cardiomyocytes

SCN5A/Nav1.5 expression is high in atrial and ventricular myocardium, His bundle, bundle branches and Purkinje fibres, but low to absent in the central sino-atrial and atrio-ventricular nodes [8]. Furthermore, *SCN5A*/Nav1.5 displays a transmural gradient in ventricular myocardium, showing higher abundance in subepicardium when compared with subendocardium (figure 1*a*) [8]. Within cardiomyocytes, Nav1.5 is localized in a distinct subcellular pattern, with a relatively low density at the crests, grooves and T-tubules at the lateral membrane (LM) versus an enrichment in the intercalated disc (ID) region (figure 1*b*) [9]. Accordingly, sodium currents measured at the ID are larger than at the LM [10], and ID-based Nav1.5 is considered especially relevant for fast propagation of electrical signals [11]. Nevertheless, loss of Nav1.5 at the LM leads to conduction slowing [12], and hence is also functionally relevant in this subcellular domain.

(c) Nav1.5 interacting proteins and macromolecular complex

Sodium channels are not isolated units within the myocyte membrane; instead, Nav1.5 forms a macromolecular complex with interacting proteins, an assortment of proteins which regulate Nav1.5 trafficking and localization as well as sodium current biophysical properties [9,13–15]. Through this complex, Nav1.5 associates not only with various isoforms of the accessory β-subunits, but also with proteins that participate in cell adhesion, signal transduction, and cytoskeleton anchoring [3,15]. Several of these Nav1.5 interacting proteins are specifically localized at or enriched in distinct subcellular domains within cardiomyocytes (figure 1*b*) [3,15]. At the LM, Nav1.5 interacts with the dystrophin–syntrophin complex, the calcium/calmodulin-dependent serine protein kinase (CASK) and caveolin-3 [16], whereas at the ID Nav1.5 associates with N-cadherin, connexin-43, β_{IV}-spectrin and desmosomal proteins such as plakophilin-2 and desmoglein-2 [15]. Nav1.5 interacting proteins likely play an essential role in the specific subcellular localization of Nav1.5 within cardiomyocytes, and their regional variation is considered to underlie at least in part the observed differences in Nav1.5 expression, sodium current density and kinetics between distinct subcellular microdomains [15]. As further discussed below, mutations in genes encoding a number of these interacting proteins have been associated with cardiac electrical disorders and/or Nav1.5 dysfunction.

(d) Transcriptional and post-translational regulation of *SCN5A* and Nav1.5

Following transcription, alternative splicing of the *SCN5A* gene produces different transcript variants. The neonatal *SCN5A*-001 transcript, which is most abundant during embryonic development, is replaced by the mature transcript *SCN5A*-003 after birth, but may be upregulated during pathophysiological conditions [17]. These two isoforms differ in exon 6 (exon 6b in *SCN5A*-003 and exon 6a in *SCN5A*-001) leading to a difference of seven amino acids and distinct gating properties of Nav1.5 [17,18]. This has been shown to be of potential functional relevance; for instance, an *SCN5A* mutation may display more severe biophysical effects in the presence of the neonatal isoform resulting in a highly malignant fetal LQTS phenotype [19]. As reviewed by Schroeter *et al.*, additional splice variants exist, displaying unique biophysical properties, tissue- and species-specific expression, and developmental regulation [17]. Following translation, Nav1.5 channels are assembled in the endoplasmic reticulum, transported to the Golgi apparatus and targeted to the membrane via the microtubule network (reviewed by Balse *et al*. [20]). Phosphorylation, glycosylation, S-nitrosylation, ubiquitination and methylation are important post-translational regulatory mechanisms impacting on Nav1.5 trafficking, function and degradation [21]. Notably, phosphorylation of Nav1.5 by PKA, PKC and calcium/calmodulin-dependent protein kinase II (CamKII) has been shown to modulate Nav1.5 trafficking as well as (late) sodium current magnitude [22,23]. Ubiquitylation of Nav1.5 mediates its internalization and subsequent degradation, and hence is an important determinant of channel density at the membrane [24]. Nav1.5 function is furthermore regulated by numerous factors, including intracellular calcium levels, reactive oxygen species and temperature [25].

(e) *SCN5A*/Nav1.5 expression in extracardiac tissue and other cell types in the heart

In addition to heart, *SCN5A* and Nav1.5 are expressed in multiple extracardiac tissues including brain, (smooth) muscle, nerves, intestine and artery (figure 1*c*). Moreover, their expression has been found in both excitable and non-excitable cell types, including SMCs, neurons, (myo)fibroblasts, endothelial cells, macrophages, thymocytes and cancer cells. The potential functional role of *SCN5A*/Nav1.5

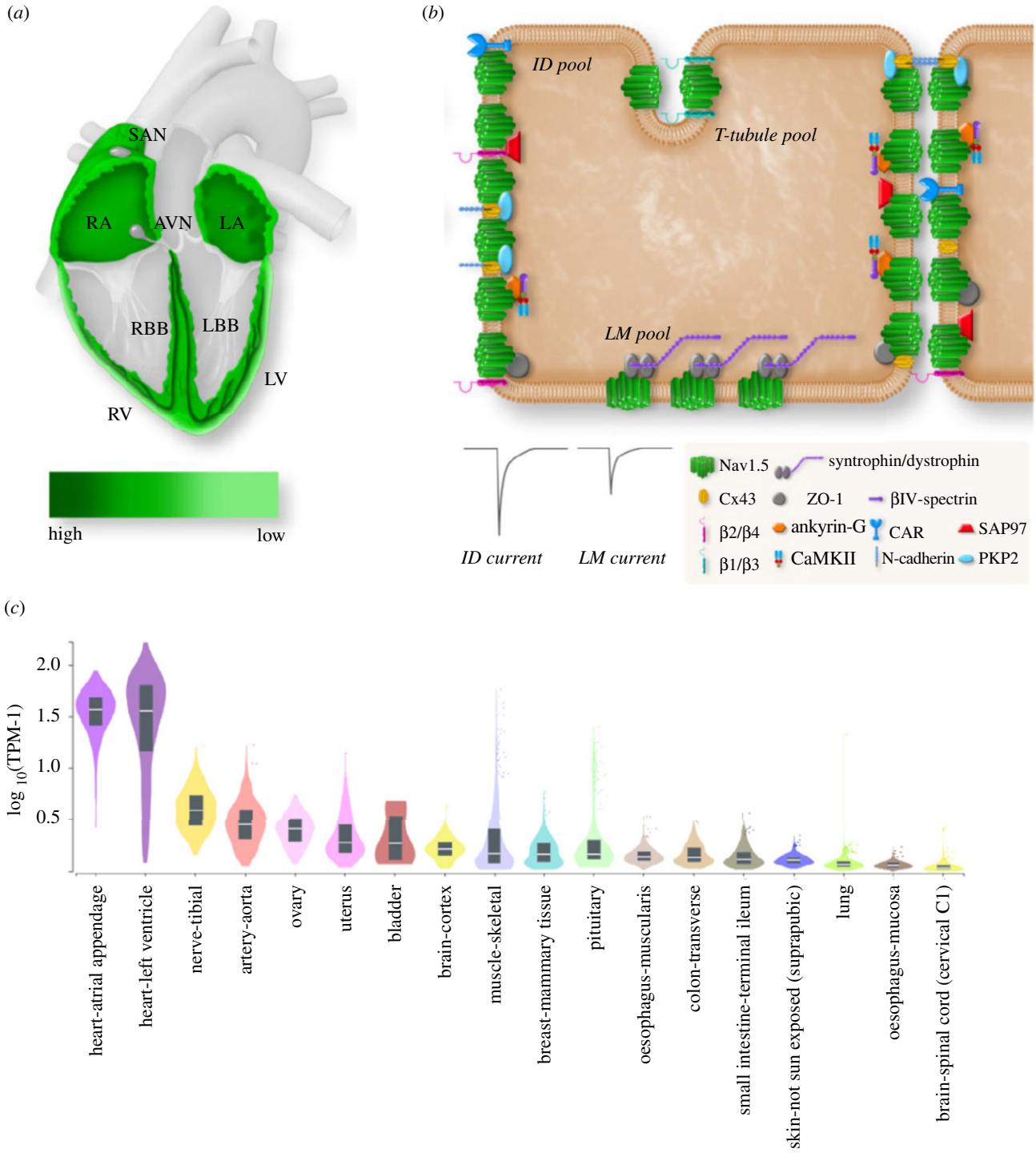

Figure 1. (*a*) Expression levels of Nav1.5 throughout various regions of the heart. (*b*) Cardiomyocyte subcellular distribution of Nav1.5 and interacting proteins in lateral membrane (LM), T-tubule and intercalated disc (ID) (from [3], with permission). (*c*) Expression levels of *SCN5A* in various human tissues (bulk tissue gene expression for ENSG00000183873.15, logarithmic scale; data source: GTEx Analysis release V8 (dbGaP accession: phs000424.v8.p2)).

in these tissues and cell types will be discussed in separate sections of this review.

3. Electrophysiological consequences of *SCN5A* dysfunction

Distinct alterations in gating and other biophysical properties of the sodium channel may have various electrophysiological consequences [26]. These can be divided into alterations causing a loss of sodium channel function and consequent depolarization disturbances, and those leading to an enhanced late sodium current and consequent prolonged repolarization (gain of function). These electrophysiological changes may, through distinct mechanisms, set the stage for potentially life-threatening ventricular arrhythmias (figure 2). The associated clinical syndromes are discussed in more detail in the next section.

(a) Causes and consequences of reduced sodium channel availability

Reduced sodium channel availability may occur in the setting of both inherited and acquired pathological conditions. Decreased peak inward sodium current and consequent lower influx of sodium ions into the cardiomyocyte reduces upstroke velocity

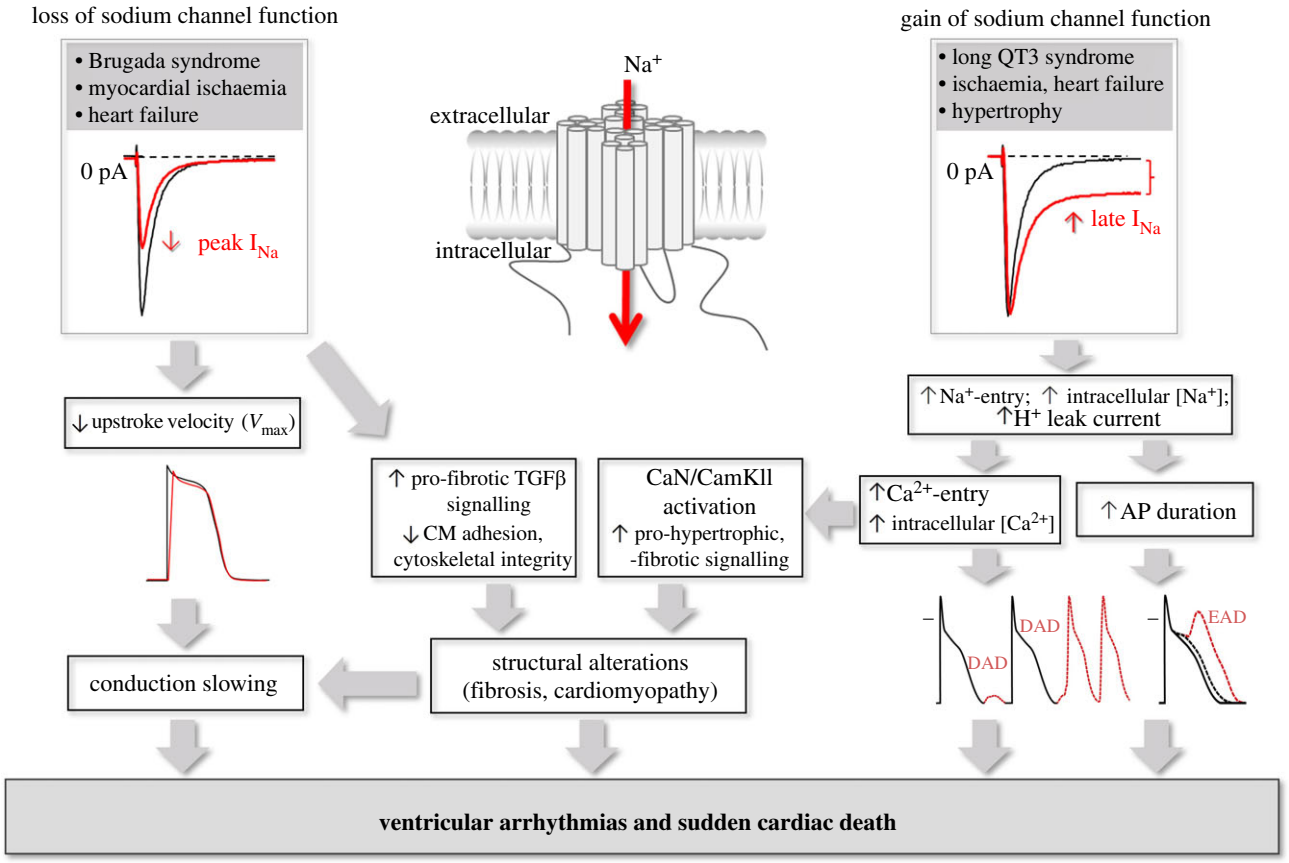

Figure 2. Pro-arrhythmic consequences of gain and loss of sodium channel function, including electrogenic effects (conduction slowing, action potential prolongation) as well as effects on cardiac structure with proposed mechanisms (CM, cardiomyocyte; CaN, calcineurin; CamKII, calmodulin-dependent protein kinase II; EAD, early afterdepolarization; DAD, delayed afterdepolarization).

of the action potential and slows propagation. Depending on the underlying cause and location involved, slowing of conduction through parts of the specialized conduction system may occur (sino-atrial node, atrio-ventricular node, His–Purkinje) and (heterogeneous) conduction slowing throughout the ventricular myocardium may set the stage for re-entrant ventricular arrhythmias. Acquired conditions include myocardial ischaemia, during which metabolic changes within the myocardium lead to inactivation of sodium channels, and heart failure, where alterations in *SCN5A* expression and post-translational regulation affect sodium current [27]. In inherited arrhythmia syndromes (see below), loss of function mutations in *SCN5A* can lead to a decreased number of functional channels on the membrane due to misfolding of the channel and/or altered trafficking. Alternatively, sodium channels may be present on the membrane with decreased functionality secondary to reduced conductivity, disruption of activation, accelerated inactivation or impaired recovery from inactivation [28,29].

(b) Causes and consequences of increased late sodium current

The initial peak sodium current is for the most part rapidly inactivated, but a small fraction of the current may persist throughout the duration of the action potential plateau phase. While this late sodium current is small during physiological conditions, it may be enhanced in the setting of acquired (ischaemia, hypertrophy and heart failure) and inherited disease (e.g. *SCN5A* mutations) [30,31]. Irrespective of the underlying cause, delayed repolarization and action potential prolongation increase the occurrence of early afterdepolarizations which can trigger torsades de pointes arrhythmias and sudden death (figure 2). Enhanced late sodium current may furthermore dysregulate intracellular sodium and calcium homeostasis: increased intracellular sodium induces a secondary rise in intracellular calcium via the sodium–calcium exchanger thereby inducing calcium-dependent pro-arrhythmic events including delayed after-depolarizations [32]. During acquired conditions such as heart failure, post-translational modulation of sodium channels by calcium-dependent pathways (such as CamKII) may increase late sodium current magnitude [33]. In the setting of inherited disorders (see below), gain of function *SCN5A* mutations often disrupt fast inactivation, allowing for sodium channels to re-open during the action potential plateau phase thereby increasing late sodium current, or alternatively may lead to slowed inactivation (resulting in channel openings of longer duration), faster recovery from inactivation, or a shift in voltage dependence of inactivation [25,34]. Certain mutations may lead to a so-called overlap syndrome, causing biophysical defects leading to both gain and loss of function of Nav1.5 (see below).

4. Disorders associated with Nav1.5 dysfunction

(a) Inherited disorders associated with *SCN5A* mutations

Mutations in *SCN5A* have been implicated in multiple inherited disorders, which each display distinct phenotypical

characteristics. These mutations may lead to a gain or a loss of sodium channel function, or a combination of both. Long QT syndrome (LQTS) is characterized by prolonged QT intervals on the ECG, and increased risk for sudden death due to ventricular tachyarrhythmias, in particular torsades de pointes. Various subtypes of LQTS exist, caused by mutations in different genes and displaying distinct clinical features. Patients with LQTS type 3 (LQT3) are often bradycardic and have a high risk of ventricular arrhythmias which occur predominantly during rest or sleep (at slow heart rates) [35]. LQT3 is caused by gain of function *SCN5A* mutations which prolong repolarization and set the stage for torsades de points arrhythmias (see previous section). Brugada syndrome (BrS) is associated with the occurrence of ventricular arrhythmias and sudden cardiac death occurring mostly in males, predominantly during rest and sleep [36]. The hallmark ECG pattern of BrS comprises a coved ST-segment in the right-precordial leads V1 to V3, which may be variably present and can be unmasked or increased after administration of sodium channel blocking drugs (ajmaline, flecainide), or during fever or exercise [36]. BrS patients frequently display low voltages, fractionated late potentials and (subtle) structural abnormalities such as fibrosis in the epicardial layer of the right ventricular outflow tract (RVOT), and arrhythmias are often inducible in the RVOT of affected individuals [37]. Loss of function *SCN5A* mutations are identified in approximately 20% of BrS patients, while mutations in other ion channel and non-ion channel genes are sporadically found [38,39]. In addition, a more complex oligogenic or polygenic inheritance is now also recognized, with multiple genetic modifiers (common or rare) defining the genetic basis [38,39]. Loss of function *SCN5A* mutations leading to reduced sodium channel availability have also been identified in patients with inherited sick sinus syndrome as well as progressive cardiac conduction defect (PCCD), also called Lenègre or Lev disease, an inherited disorder characterized by progressive conduction slowing through the His–Purkinje system, (complete) AV block, syncope and sudden death [40,41]. Mutations in *SCN5A* have furthermore been associated with atrial fibrillation in young patients with structurally normal hearts. Both loss of function and gain of function mutations have been described in this familial form, which may induce AF by decreasing atrial conduction velocity and/or increasing atrial excitability [42,43]. In some instances, one single *SCN5A* mutation can result in a 'sodium channel overlap syndrome', with multiple disease phenotypes consistent with both gain and loss of sodium channel function occurring even within one affected family. For instance, carriers of the *SCN5A*-1795insD mutation within in a large Dutch family present with extensive variability in type and severity of symptoms, including sinus node dysfunction, bradycardia, conduction disease, BrS, LQT3 (either in isolation or in combinations thereof), and an increased risk for nocturnal sudden death [44]. Electrophysiological analysis of transgenic mice carrying the heterozygous *Scn5a*-1798insD$^{/+}$ mutation (the mouse homologue of the human mutation) demonstrated that the mutation caused a reduced peak sodium current density, a delayed time course of fast inactivation and a small late sodium current, explaining the observed multiple phenotypes [45]. Similar clinical and electrophysiological overlap has been reported for other *SCN5A* mutations, although a clear parallel between the mixed clinical phenotype of a certain *SCN5A* mutation and its biophysical properties is not always observed; here, factors such as age and genetic modifiers may play a modulatory role [46,47]. Finally, *SCN5A* mutations are increasingly associated with dilated and arrhythmogenic cardiomyopathy (ACM), which will be discussed in more detail in the section below.

Table 1. Nav1.5 interacting proteins, their (preferential) localization in adult cardiomyocytes (ID, intercalated discs; LM, lateral membrane), and associated inherited cardiac disorders (LQTS, long QT syndrome; BrS, Brugada syndrome; AF, atrial fibrillation; CCD, cardiac conduction disease; SND, sinus node dysfunction; CPVT, catecholaminergic polymorphic ventricular tachycardia; IVF, idiopathic ventricular fibrillation; ACM, arrhythmogenic cardiomyopathy; DCM, dilated cardiomyopathy).

gene	protein	subdomain	associated cardiac disease
SCN1B	β1	LM, ID	BrS, AF, CCD
SCN2B	β2	ID	AF
SCN3B	β3	LM	BrS, AF
SCN4B	β4	LM, ID	LQTS, AF
SNTA1	α1-syntrophin	LM	LQTS
CASK	calcium/calmodulin-dependent serine protein kinase (CASK)	LM	–
ANK2	ankyrin-B	LM	LQTS, SND, CPVT, IVF
CAV3	caveolin-3	LM	LQTS
FGF13	fibroblast growth factor 13	LM, ID	–
ANK3	ankyrin-G	LM, ID	–
PKP2	plakophilin-2	ID	ACM
DSG2	desmoglein-2	ID	ACM, DCM
CDH2	N-cadherin	ID	ACM
SPTBN4	β_{IV}-spectrin	ID	–
DCTN2	dynactin subunit 2 (p50/dynamitin)	ID	–
SAP97	SAP97; DLG1	ID	–
GPD1L	glycerol-3-phosphate dehydrogenase 1-like	unknown	BrS

(b) Arrhythmia disorders associated with mutations in Nav1.5-interacting proteins

As mentioned above, Nav1.5 forms part of a macromolecular complex through which it interacts with a large number of associated proteins. Crucially, mutations in these interacting proteins have been associated with sodium channel dysfunction and arrhythmia syndromes (table 1). For instance, mutations in the sodium channel accessory β-subunits have been identified in patients with BrS, LQTS, atrial fibrillation and idiopathic ventricular fibrillation [46]. Furthermore, mutations in the Nav1.5 interacting proteins alpha-1-syntrophin and caveolin-3, which are located at the LM,

have been reported in LQTS patients and have been demonstrated to increase late sodium current [48,49], while mutations in ankyrin-B have been associated with sinus node dysfunction, idiopathic ventricular fibrillation, LQTS and catecholaminergic polymorphic ventricular tachycardia [50,51]. Moreover, the absence of dystrophin in Duchenne muscular dystrophy is associated with arrhythmias in affected patients and a reduced sodium current is observed in *mdx* mouse cardiomyocytes [52]. Finally, mutations in genes encoding desmosomal proteins located at the ID region have been associated with ACM [53]. Crucially, a number of these proteins interact with Nav1.5 and decreased sodium current has been observed in ACM models, even prior to development of cardiomyopathic changes [54,55]. Interestingly, as discussed in more detail below, *SCN5A* mutations have now also been implicated in ACM.

(c) Acquired disorders associated with Nav1.5 dysfunction

As briefly mentioned above, in addition to inherited disorders, sodium channel dysfunction also occurs in the setting of a number of acquired conditions. Apart from alterations in (late) sodium current and consequent deleterious effects on conduction, repolarization and calcium homeostasis in the setting of myocardial ischaemia, Nav1.5 dysfunction is also observed in metabolic disorders such as diabetes and obesity [56]. Here, both loss and gain of sodium channel function have been observed; a reduced *SCN5A* expression and smaller peak sodium current leading to conduction slowing [57], as well as enhanced late sodium current. The latter is the consequence of metabolic dysregulation, including alterations in cytosolic calcium and CaMKII [58,59], and may ultimately lead to the occurrence of calcium-dependent pro-arrhythmic events. Furthermore, a crucial role for Nav1.5 has been described in tumour growth, invasiveness and metastasis of certain cancers; this topic will be discussed in more detail in a later section of this review.

5. Cardiomyopathy and *SCN5A* channelopathy: mechanisms and consequences

(a) *SCN5A* channelopathy, cardiac structural alterations and cardiomyopathy

In addition to primary electrical disorders, *SCN5A* mutations have also been associated with cardiac structural alterations such as myocardial fibrosis and (dilated) cardiomyopathy [60–64]. In BrS patients, ventricular hypertrophy has been reported in addition to increased collagen content and fibrosis, particularly in the subepicardium of the RVOT [64]. Furthermore, familial forms of dilated cardiomyopathy (DCM) have been reported in patients with *SCN5A* mutations, often presenting in combination with conduction disease, atrial arrhythmias and/or fibrillation [62,65]. Interestingly, biophysical properties consistent with both loss and gain of sodium channel function have been observed in *SCN5A* mutations associated with DCM [65,66]. A specific subtype, characterized by DCM and multifocal ectopic Purkinje-related premature contractions (MEPPC), is associated with gain of function *SCN5A* mutations which lead to hyperexcitability of the fascicular–Purkinje system [67,68]. *SCN5A* mutations are now increasingly identified in DCM patients [69], and can present with a broad range of electrical disorders ranging from conduction abnormalities to atrial and ventricular arrhythmias [70]. More recently, an overlap between BrS and arrhythmogenic (right ventricular) cardiomyopathy (ARVC/ACM) has been described; clinical features of both syndromes were observed in patients that did not carry mutations in ARVC/ACM-related desmosomal proteins, but were found to have a (loss of function) *SCN5A* mutation [38,53]. ARVC and BrS both affect predominantly the right ventricle with involvement of cardiac cell–cell junctions, and demonstrate pathophysiological, genetic, structural and electrophysiological overlap [71]. While cardiac structural alterations were originally considered to occur secondary to repetitive, long-standing arrhythmias in affected patients, they are now increasingly recognized as a more direct consequence of Nav1.5 dysfunction in the setting of *SCN5A* mutations. This is further strengthened by the age-dependent development of structural remodelling in mice with heterozygous *Scn5a* deficiency [72]. Clearly, development of cardiac structural derangements will contribute to arrhythmogenesis in affected patients. For instance, in the *SCN5A*-1795insD family, we found that most mutation carriers who suffered ventricular arrhythmias above the age of 40 years (despite pacemaker treatment), had ventricular hypertrophy [44,73]. Although the latter could be explained by hypertension in these patients, studies in mice carrying the mouse homologue mutation demonstrated age-dependent development of hypertrophy and fibrosis, indicating a direct effect of Nav1.5 dysfunction, as discussed in more detail below [44].

(b) *SCN5A*/Nav1.5 and cardiac development

The observation that both gain and loss of function mutations are associated with cardiomyopathy suggests that Nav1.5 may also impact on cardiac structure in a non-electrogenic manner, independent from its ion-conducting properties. Nav1.5 is present at early developmental stages but is not yet capable of generating sodium currents because of the relatively depolarized resting membrane potential [74]. In zebrafish, the presence of *Scn5a* homologues was found to be essential for normal cardiac development at an early stage, before sodium channels impact on the electrophysiological properties of the heart; pharmacological inhibition of sodium current did not have the same effect, indicating that the early embryonic lethality occurred independently of electrogenic effects of Nav1.5 [75]. Similarly, *Scn5a*-deficient and mutant mice die at an early embryonic stage [76,77], and we recently demonstrated abnormal development and cardiac structural abnormalities in homozygous *Scn5a*-1798insD embryos at an early stage before sodium channels become functionally relevant for cardiac electrical activity, providing further evidence for a non-electrogenic role for Nav1.5 [78].

(c) Mechanisms underlying cardiac structural alterations secondary to Nav1.5 dysfunction

Cardiac structural remodelling secondary to *SCN5A* mutations may be explained by various mechanisms (figure 2). In the setting of gain of function mutations, enhanced late sodium current and consequent increased

sodium influx will result in elevated intracellular calcium levels by way of the sodium–calcium exchanger. Increased calcium concentrations may activate calcium/calmodulin-dependent protein kinase II (CAMKII) and protein phosphatase calcineurin (CaN), signalling pathways controlling pro-hypertrophic and pro-fibrotic gene transcription [3,79]. This has indeed been confirmed in studies in murine LQT3 models, which have also demonstrated the beneficial effects of the late sodium current inhibitor ranolazine on calcium homeostasis, pro-arrhythmia and cardiac structural alterations [32,44,80,81]. Certain DCM-related *SCN5A* mutations have been shown to induce a proton leak current which leads to intracellular sodium overload via the sodium–hydrogen exchanger, ultimately resulting in intracellular calcium overload [82,83]. Ionic imbalances and intracellular acidosis may not only impact on arrhythmogenesis and pro-fibrotic pathways, but also potentially impair excitation–contraction and myofilament function [83]. With respect to structural consequences of loss of sodium channel function, electrical activity-dependent stimulation of pro-fibrotic factors of the transforming growth factor β (TGFβ) pathway has been proposed [84], with inhibition of sodium current leading to increased levels of TGFβ1 receptors [85]. Interestingly, both fibrosis and TGFβ1 transcript levels were found to be increased in ventricles of aged heterozygous *Scn5a*-deficient mice, suggesting development of TGFβ1-mediated structural abnormalities secondary to sodium channel dysfunction [86]. A potential non-electrogenic mechanism of loss of function *SCN5A* mutations on cardiac structure involves protein–protein interactions. As described in previous sections, Nav1.5 interacts with a variety of proteins, including cytoskeletal proteins, components of the extracellular matrix and adhesion- and desmosomal proteins at the ID. The latter function as mechanical linkers between cardiomyocytes, and are vital for myocardial structure. It is therefore tempting to speculate that Nav1.5 dysfunction impairs the function or localization of these mechanical linkers or contractile proteins, destabilizing intercellular adhesion and/or cytoskeletal integrity. Indeed, loss of Nav1.5 in HL1 cells has been shown to reduce intercellular adhesion strength [87]. However, while it is clear that Nav1.5 interacting proteins modulate sodium channel function [9,13–15], there is currently limited experimental evidence to support the notion that modulation also occurs in the opposite direction, and the impact of different *SCN5A* mutations on interacting proteins remains to investigated. Overall, it is clear that Nav1.5 not only determines electrophysiological characteristics of the myocardium, but also exerts effects on myocardial structure and function through various mechanisms (figure 2).

6. Role of *SCN5A*/Nav1.5 in cancer

(a) Impact of *SCN5A*/Nav1.5 on tumour growth and metastasis

Increased expression of sodium channels in tumours is well established, with different sodium channel isoforms found in distinct tumour types [88]. Nav1.5 is present in many types of tumour tissue, including breast, colon, lymphoma, neuroblastoma, non-small cell lung cancer, ovarian, small cell lung cancer, with the highest overexpression levels observed in metastatic breast cancer, colon cancer and ovarian cancer [89,90]. Moreover, Nav1.5 represents the most highly expressed subunit in lymphoma and breast cancer cells [90]. While the adult isoform of *SCN5A* is found in colon cancer cells, in breast and prostate cancer cells the neonatal *SCN5A* splice variant is mostly expressed [89,90]. Overall, increased expression of Nav1.5 is associated with more aggressive tumour characteristics, including lymph node invasion, recurrence of metastasis and reduced survival [91]. This has been observed for breast cancer, colon cancer and ovarian cancer, but not for gliomas or lung cancer cells [90]. Silencing either adult or neonatal *SCN5A* has been shown to reduce invasion and migration of MDA-MB-231 breast cancer cells [92,93], and MDA-MB-231 cells with downregulated Nav1.5 orthotopically implanted into mice showed reduced tumour growth and local invasion *in vivo* [94].

(b) Mechanisms underlying Nav1.5-mediated alterations in tumour growth and metastasis

Nav1.5 regulates a number of processes in tumour cells which can affect its metastatic properties, including galvanotaxis (directed movement in response to electrical current), migration, invasion, adhesion, and gene expression [90]. In tumour cells, sodium channels are particularly enriched in invadopodia, plasma membrane protrusions involved in extracellular matrix degeneration and therefore relevant for migration and invasion [95,96]. In breast cancer cells, Nav1.5 channels co-localize and coimmunoprecipitate with the nitrogen–hydrogen exchanger 1 (NHE-1) and increase its activity [95,97,98], enhancing influx of sodium and efflux of hydrogen. This results in acidification (and thus a lower pH) of the extracellular environment and an alkalization of the intracellular environment. This acidification of the extracellular space increases cathepsin activity, enhances invadopodial formation and activity and promotes invasion through extracellular matrix degeneration, enabling the tumour to metastasize [95,97]. Increased expression of (neonatal) Nav1.5 in cancer cells may also lead to increased late sodium current and subsequent increased intracellular calcium levels, which in turn may activate SNARE-mediated vesicle fusion and stimulate the activity of podosomes, actin-rich plasma membrane components that facilitate cell motility, adhesion and migration of tumour cells [90,97,99]. Indeed, abnormally high sodium concentrations are found in several types of cancer cells [100], and non-invasive imaging of sodium accumulation in breast tissue (^{23}Na MRI) may provide a useful diagnostic and prognostic biomarker [101].

(c) Therapeutic considerations

Given the deleterious effects of overexpression of Nav1.5 in tumour cells, pharmacological inhibition of Nav1.5 would be expected to be beneficial. Indeed, tetrodotoxin (TTX) has been reported to inhibit Nav1.5, impeding sodium influx, phagocytosis and endosomal acidification in breast cancer cells and reducing their invasion and migration [95]. Similarly, Nav1.5 blockade with phenytoin reduced invasion of breast cancer cells *in vitro* [91], and reduced tumour growth, invasion, proliferation and metastasis of breast cancer cells *in vivo* in mice [102]. In addition, ranolazine inhibited Nav1.5-mediated breast cancer cell invasiveness *in vitro* and attenuated metastatic lung colonization by breast cancer cells in mice [103]. It is important to note that,

since the expression of sodium channel isoforms other than Nav1.5 is also increased in certain types of cancer cells [88], (part of) the observed effects of sodium channel blockers may also be due to their action on these other isoforms. Theoretically, patients using such sodium channel blocking drugs (for instance, for epilepsy) who subsequently develop cancer, may have a less aggressive tumour with less extensive metastasis. However, such a beneficial effect has as yet not been observed [104], and prospective studies will be required to investigate this further. Moreover, it should be noted that the use of sodium blocking drugs carries a potential risk of side effects, including cardiac conduction slowing and arrhythmias. More recently, small molecule blockers of neonatal Nav1.5 were designed, which reduced invasion of breast cancer cells *in vitro*, providing a promising novel therapeutic approach [105].

7. *SCN5A*/Nav1.5 (dys)function in brain and neuronal tissue

(a) *SCN5A*/Nav1.5 expression in neuronal tissue

Neuronal tissues express a wide range of sodium channel isoforms displaying distinct biophysical properties and TTX sensitivity, and their differential expression have been shown to mediate neuron subtype-specific firing patterns [106]. In addition to neuronal sodium channel isoforms, several studies have also detected the presence of *SCN5A* and Nav1.5 in rodent and human brain, ganglia and neurons. In the mouse brain, Nav1.5 protein has been observed in the cortex, thalamus, hypothalamus, basal ganglia, cerebellum and brain stem, clustering in axons [107]. In the frontal lobe cortex of the human brain, Nav1.5 was found to be predominantly located in neuronal cell bodies, axons and dendrites, but to a far lesser extent in glial components [108]. On the mRNA level, *SCN5A* showed a restricted expression pattern in rat forebrain with selective localization in limbic regions, including the pirifom cortex and subcortical limbic nuclei [109]. These regions include projections to and from the amygdala, hippocampus and hypothalamus, indicating that *SCN5A* may play a role in or modulate various functions such as olfactory perception and autonomic responses [109]. *SCN5A*/Nav1.5 expression has furthermore been observed in dorsal root ganglia (DRG), vestibular ganglion neurons, olfactory sensory neurons and intracardiac neurons [110–113], with electrophysiological studies indicating a functional role for Nav1.5 in neuronal firing in a number of these tissues [110,112]. Crucially, distinct *SCN5A* splice isoforms appear relevant for neuronal function. For instance, in mouse DRG the exon 18-deleted Nav1.5a is the predominant isoform, which has a 53-amino acid truncation in the intracellular loop between DII and III and consequently altered inactivation properties [110]. By contrast, wild-type Nav1.5 in addition to the splice variants Nav1.5c (containing the variant Q1077) and Nav1.5e (the embryonic or neonatal variant containing exon 6A instead of 6) were found to be expressed in human brain cortex [108]. While the overall expression of *SCN5A*/Nav1.5 in neuronal tissue is clearly lower than in the heart, their increasingly reported involvement in several neurological conditions underscores their functional relevance in the brain and neurons.

(b) *SCN5A* and epilepsy

Mutations in various ion channels are well known to be associated with various forms of epilepsy. It is, however, becoming increasingly clear that patients with inherited cardiac arrhythmia syndromes such as LQTS may present with seizures, and they are often (initially) misdiagnosed with epilepsy [114]. In a cohort of 610 LQTS patients, 11% displayed seizures or seizure-like episodes, the majority of which had a diagnosis of LQTS2 associated with a mutation in *KCNH2* [115]. Nevertheless, in addition to mutations in neuronal sodium channels, a potential role for *SCN5A*/Nav1.5 in epilepsy has been described. In a rat model of temporal lobe epilepsy, increased expression of *Scn5a* and Nav1.5 was observed in the brain, including the hippocampus [116]. In addition, a number of case reports and studies have described the identification of *SCN5A* mutations in patients with epilepsy, mostly co-occurring with other *SCN5A*-related cardiac disorders. Epileptic seizures have been reported as the initial clinical presentation in patients with BrS [117,118]. In one family, three males carrying the *SCN5A*-p.W1095X mutation were initially treated for epileptic seizures in childhood before being diagnosed with BrS during adulthood [119]. In a child with LQTS and neonatal seizures, the *SCN5A* mutation p.R1623Q was found, which is known to be associated with a severe LQTS phenotype [120]. In a further study of 21 infants with LQTS, a high incidence of epilepsy and neurodevelopmental disorders was observed in patients with perinatal LQTS, whereas this did not occur in those with non-perinatal LQTS [121]. Of the perinatal LQTS patients suffering epileptic seizures, two patients carried *SCN5A* mutations (G1631D and P1332L) [121]. In an infant presenting with QT-prolongation and self-terminating torsades de pointes shortly after birth, a generalized tonic–clonic seizure (in the setting of a normal heart rhythm) occurred 2 days after birth, and the infant was found to carry the *SCN5A*-M1766L mutation [122]. While hypoperfusion of the brain consequent to arrhythmias may increase susceptibility to epilepsy in LQTS patients, some reports demonstrated the occurrence of seizures in the absence of cerebral abnormalities, indicating a more direct mechanistic link [121]. From a biophysical point of view, gain of function *SCN5A* mutations may lead to persistent depolarization of neurons, increased firing frequency and potentially epileptiform bursting behaviour; in contrast, it is difficult to predict how loss of function mutations observed in BrS could lead to seizures. Interestingly, as indicated above, *SCN5A* is expressed in limbic regions such as the piriform cortex; the latter area is known to have a low threshold for epileptogenesis [109]. Nevertheless, functional proof of a direct link between *SCN5A* mutations and epilepsy is as yet still lacking.

(c) *SCN5A* and sudden unexpected death in epilepsy

In recent years, it has become increasingly clear that epilepsy patients are at increased risk of sudden death, a condition known as sudden unexpected death in epilepsy (SUDEP), with the incidence of SUDEP in the general epilepsy population being around 1.2–1.3 per 1000 person-years [123–125]. SUDEP is defined as 'sudden, unexpected, witnessed or unwitnessed, non-traumatic and non-drowning death, occurring in benign circumstances, in an individual with epilepsy, with or without evidence for a seizure and excluding documented status epilepticus (seizure duration ≥30 min or seizures without recovery in between), in which post-mortem examination

does not reveal a cause of death' [126]. Clinical risk factors for SUDEP include recent seizures, increased seizure frequency and nocturnal seizures [124,126]. The pathophysiology of SUDEP is likely heterogeneous and includes ictal and peri-ictal respiratory dysfunction and apnoea, autonomic nervous system dysregulation, arousal system dysfunction, neurotransmitter dysbalance and ion channel dysfunction [125–127]. SUDEP may occur during or immediately following a generalized tonic–clonic seizure, but is also observed without any immediately prior visible seizures, indicating a potential role for other pathomechanisms, including cardiac [126]. Indeed, the genetic spectrum of SUDEP not only includes epilepsy genes and genes involved in respiration and arousal, but variants in arrhythmia genes are also increasingly recognized, including *KCNH2*, *KCNQ1*, *RYR2* and *SCN5A* [128,129]. Genetic testing using post-mortem blood collected from 48 definite or possible SUDEP cases revealed, in addition to variants in *KCNQ1* and *KCNH2*, six synonymous and three non-synonymous variants in *SCN5A*; the latter were all previously associated with LQTS [129]. In another cohort of 61 SUDEP cases, two *SCN5A* variants (Ile397Val and Val223Gly) were identified [130]. Interestingly, Ile397Val and Val223Leu (but not Val223Gly as in the SUDEP case) were previously reported in BrS patients [29,131]. A case report furthermore described a female patient diagnosed with epilepsy but who died 1 year later in her sleep; post-mortem genetic analysis revealed the presence of the *SCN5A*-R523C mutation [132], which was previously reported in an LQTS patient [133]. To gain further insight into the potential pathogenicity of these last three variants, Soh and colleagues investigated their biophysical properties in CHO cells and observed a reduced peak current for Val223Gly as well as changes in gating properties for all three variants, underlining their potential functional impact [134]. Hence, the (co-)occurrence of *SCN5A* mutations in these patients may have contributed to the development of cardiac arrhythmias and potentially SUDEP. However, some of these patients were also treated with anti-epileptic drugs, many of which are in fact sodium channel blockers, which may by themselves, or in co-occurrence with *SCN5A* variants, decrease peak sodium current and increase the risk for arrhythmias [135,136], thereby providing an additional factor underlying and/or contributing to SUDEP.

(d) *SCN5A* and other neurological disorders

In addition to epilepsy, *SCN5A* and Nav1.5 have been implicated in other neurological disorders. The expression of *SCN5A*/Nav1.5 was found to be downregulated in DRG neurons and axons of peripheral sensory neurons in rats with spared nerve injury, a model of neuropathic pain [137], but the functional implications of this observation remains unknown. A potential role for *SCN5A* in schizophrenia has also been proposed, possibly by altering the activity of the limbic system [138]. Interestingly, Blom and colleagues observed an increased prevalence of a Brugada ECG pattern in patients with schizophrenia when compared with similarly aged controls [139]. While these findings suggest a higher prevalence of BrS in schizophrenia patients (which in turn also have an increased risk for SCD), other factors may also play a confounding role. For instance, drugs used in the management of schizophrenia may also block cardiac sodium channels, and as such increase the risk for arrhythmias [140]. However, the observed increased prevalence of a Brugada ECG was independent of sodium channel blocker use [139], suggesting a more direct interaction. Indeed, ion channels have been implicated in mediating schizophrenia risk [141], and hence further investigation of a potential role for *SCN5A* is warranted. Finally, alterations in Nav1.5 have been demonstrated in multiple sclerosis (MS), a condition associated with destruction of myelin sheaths and axonal damage. Upregulated Nav1.5 expression was observed in and around MS lesions, in particular within reactive astrocytes, which play a prominent role in the response of the nervous system to injury [142]. This upregulation was proposed to provide a compensatory mechanism in order to maintain ionic homeostasis and prevent injury-induced calcium dysregulation [142,143]. Indeed, deletion of Nav1.5 from astrocytes in mice significantly worsened outcomes in an experimental model of MS, with increased inflammatory infiltrate in both early and late stages of the disease [144]. Nav1.5 expression was furthermore observed in macrophages within active MS lesions (predominantly in late endosomes and phagolysosomes), suggesting a potential role in phagocytic myelin degradation [145].

8. *SCN5A* channelopathies and gastrointestinal disorders

(a) Expression and function of Nav1.5 in the gastrointestinal tract

Interstitial cells of Cajal (ICC) and intestinal SMCs are essential for gastrointestinal motility. Smooth muscle contractility is governed by excitability and changes in membrane potential, and as such is regulated by a wide variety of ion channels, including sodium channels [146]. Of the latter, Nav1.5 is the predominant isoform in the gastrointestinal tract, with functional expression of *SCN5A*/Nav1.5 having been demonstrated in rat and human jejunal and colon circular SMCs [147–149]. While calcium is classically considered key for SMC function, a role for sodium channels is increasingly recognized [150]. Nav1.5 activation may impact on gastrointestinal motility by modulating electrical slow waves in ICC, and by regulating calcium entry and subsequent contraction in SMC [150,151]. Crucially, Nav1.5 in ICC is mechanosensitive: it is activated by shear stress, with stretch increasing slow wave frequency in human ICC, while sodium current inhibition by lidocaine decreased slow wave frequency [151]. Moreover, ranolazine was found to decrease peak sodium current and prevent shear stress-induced increase in sodium current in human colon SMCs, in addition to reducing contractility of human colon muscle strips [148]. The latter observation has been suggested to underlie the known side-effect of obstipation reported with use of ranolazine [148].

(b) Gastrointestinal disorders associated with *SCN5A* mutations

In 2006, the first potential association between *SCN5A* mutations and gastrointestinal disorders was presented. Of 31 *SCN5A* mutation carriers, more than half self-reported abdominal pain and/or other gastrointestinal symptoms; most of these patients had a gain of function mutation and

a clinical LQT3 phenotype [152]. Given the localization of Nav1.5 in the gastrointestinal tract, and the fact that a genetic basis has been proposed for irritable bowel syndrome (IBS), Saito *et al*. investigated the presence of *SCN5A* mutations in 49 IBS patients [153]. In one patient, they identified the *SCN5A*-G298S mutation which was shown to lead to a loss of sodium channel function [153]. Subsequent studies in larger IBS cohorts confirmed this initial observation, reporting the presence of an *SCN5A* mutation in approximately 2% of IBS patients [154,155], with the majority of *SCN5A* mutation carriers suffering from constipation-predominant IBS [154]. Most *SCN5A* mutations identified in IBS patients have been shown to lead to loss of sodium channel function [154,155]. The *SCN5A*-A997T mutation, identified in a patient suffering from constipation-predominant IBS, was found to almost completely abolish peak sodium current, which could be rescued by chronic incubation with mexiletine; interestingly, mexiletine treatment of the patient successfully reduced IBS symptoms [154]. In addition, identified mutations also impaired mechanosensitive function of Nav1.5 [155]. In fact, the IBS-associated mutation *SCN5A*-G615E was observed to have limited impact on baseline sodium current function, but mostly caused disruptions in mechanosensitivity and mechano-electrical feedback [156]. While this observation may suggest that reduced mechanosensitivity underlies smooth muscle pathophysiology caused by *SCN5A* mutations, it will require further mechanistic investigation.

9. *SCN5A*/Nav1.5 (dys)function, immune response and inflammation

(a) *SCN5A*/Nav1.5 in macrophages

In 2007, work by Carrithers *et al*. revealed the presence of Nav1.5 on the late endosome of human monocyte-derived macrophages [157]. They furthermore demonstrated a functional role for Nav1.5 in regulating phagocytosis and endosomal acidification, providing evidence that lipopolysaccharide-induced activation of Nav1.5 leads to sodium efflux and consequently decreased intraendosomal pH [157]. A subsequent study by the same group showed that Nav1.5 also regulates phagocytosis and intracellular processing of mycobacteria by human macrophages [158]. Human macrophage *SCN5A* was furthermore found to act as a pathogen sensor, initiating signalling and transcription of immune response genes for antiviral host defence, enhancing the anti-inflammatory profile of macrophages [159]. More recently, it was concluded that human macrophage *SCN5A* mediates an innate immune signalling pathway that limits DNA damage through increased expression of *PPP1R10*, a regulator of phosphatase activity and DNA repair [160]. As discussed in a previous section, Nav1.5 expression has been found in late endosomes and phagolysosomes of macrophages within active MS lesions, where they likely participate in the phagocytic pathway of myelin degradation [145]; moreover, transfer of macrophages expressing Nav1.5 into mice with experimentally induced autoimmune encephalomyelitis promoted recovery [161].

(b) *SCN5A*/Nav1.5 in thymocytes and T cells

Thymocytes, immune cells in the thymus, transform into mature T cells through a selection process by which peripheral T cells are formed that are able to respond to foreign pathogens but do not target the body's own antigens. Lo and colleagues demonstrated that *SCN5A* mediates calcium entry into $CD4^+CD8^+$ double-positive thymocytes, thereby regulating positive selection of $CD4^+$ T cells in the thymus [162]. Moreover, they showed that overexpression of *SCN5A* in peripheral T cells resulted in altered $CD4^+$ T-cell sensitivity and T-cell receptor signalling, in addition to an impaired response during infection [163]. Since *SCN5A* is normally no longer detectable in T cells following the $CD4^+$ T-cell selection stage, the authors hypothesized that this repression of *SCN5A* is essential to prevent unwanted T-cell response and consequent auto-immunity [163].

(c) Potential implications for arrhythmogenesis

Macrophages may play an important role in arrhythmogenesis via a number of mechanisms, including secretion of pro-inflammatory cytokines, alterations in cell-to-cell coupling, and induction of electrical, structural and autonomic remodelling [164]. Hence, alterations in macrophage function consequent to alterations in *SCN5A* may well lead to an abnormal immune response, autoimmune disorder and ultimately pro-arrhythmia. Although there is as yet little direct evidence for such a disease mechanism, signs of myocardial inflammation have been reported in a large proportion of BrS patients, in particular in the RVOT [165]. Moreover, autoantibodies directed at a number of cardiac proteins have been detected in plasma samples of BrS patients [166]. Recently, extensive myocardial inflammation in the absence of myocarditis or sarcoidosis was found in a patient who was successfully resuscitated for ventricular fibrillation, and who was found to carry a novel likely pathogenic *SCN5A* variant [167]. Moreover, myocardial inflammation is commonly observed in patients with ACM [53], which has been associated with *SCN5A* variants (see earlier sections).

10. Other non-cardiomyocyte effects of *SCN5A*/Nav1.5

(a) *SCN5A*/Nav1.5 in (vascular) smooth muscle cells

As discussed in the previous section, Nav1.5 is expressed in SMCs of the gastrointestinal tract where they are thought to regulate motility. Some studies have also reported its (functional) presence in SMCs of other tissues, where they may contribute to the generation and/or propagation of spontaneous electrical activity underlying myogenic contractions [168]. *SCN5A* expression and functional Nav1.5 activity was detected in airway SMCs from rabbit bronchi [168]. Although the authors were not able to demonstrate a potential impact on myogenic contraction, they hypothesized that this is likely due to the fact that Nav1.5 channels are inactivated at the relatively depolarized membrane potential of these cells under normal conditions, but that they may still become functionally relevant during pathophysiological conditions and contribute to e.g. bronchospasm [168]. In rat femoral artery, Nav1.5 was found to be expressed in endothelial cells and SMCs of the media [169]. Exposure to the Nav1.5 activator veratridine or hypoxia caused vasocontraction, which was prevented by the potent Nav1.5 channel blocker F-15845; SMCs rather than endothelial cells were shown to be involved in these effects [169]. In bovine pulmonary artery SMCs, Nav1.5 expression was observed in the cell

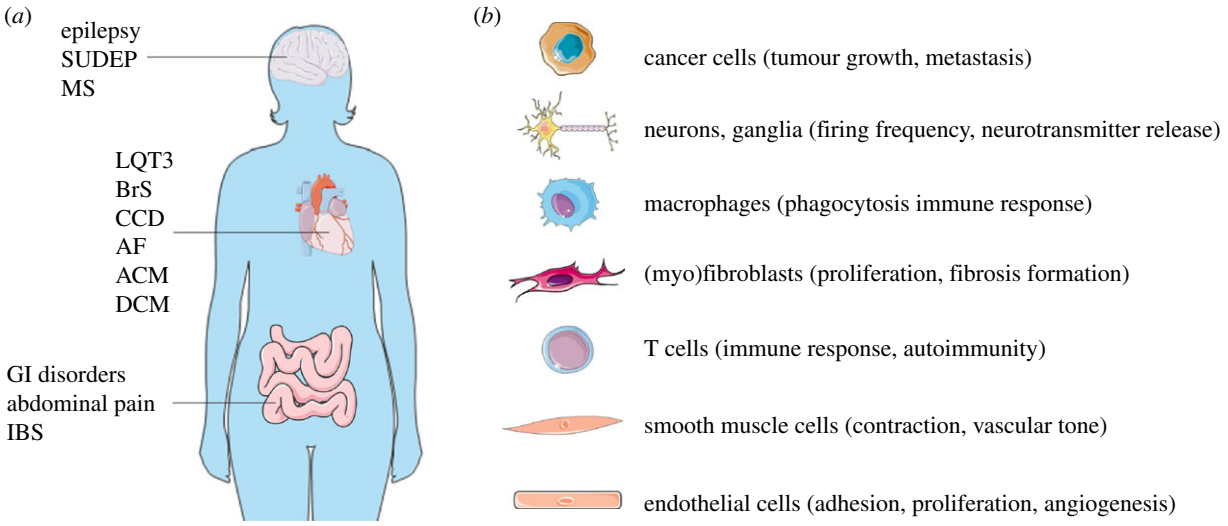

Figure 3. (*a*) Disorders in brain, heart and intestine associated with *SCN5A* mutations/variants (SUDEP, sudden unexplained death in epilepsy; MS, multiple sclerosis; LQT3, Long QT syndrome type 3; BrS, Brugada syndrome; AF, atrial fibrillation; CCD, cardiac conduction disease; ACM, arrhythmogenic cardiomyopathy; DCM, dilated cardiomyopathy; GI, gastrointestinal; IBS, irritable bowel syndrome). (*b*) Non-cardiomyocyte cell types expressing *SCN5A*/Nav1.5 with proposed function.

membrane and caveolae, where they were shown to mediate the effects of the vasoconstrictor endothelin-1 on pulmonary vascular tone [170]. Taken together, these studies suggest a potential modulatory role of Nav1.5 in SMCs with putative functional impact on various (patho)physiological processes, but the evidence is as yet limited and further exploration is warranted.

(b) *SCN5A*/Nav1.5 in endothelial cells

In cultured endothelial cells derived from rat interlobar artery and human umbilical vein, a TTX-resistant sodium current was identified with properties similar to Nav1.5 [171]. Similarly, a TTX-resistant sodium current was observed in endothelial cells cultured from human saphenous vein, in addition to expression of *SCN5A* [172]. As mentioned above, Nav1.5 was also found to be expressed in endothelial cells of rat femoral artery, but no functional impact was observed [169]. Human umbilical vein endothelial cells (HUVECs) express both Nav1.5 and Nav1.7 and display a mostly TTX-resistant current; inhibition of the latter (i.e. inhibition of Nav1.5) inhibited HUVEC angiogenesis activity, increased HUVEC adhesion, and reduced HUVEC proliferation induced by vascular endothelial growth factor (VEGF) [173]. Moreover, Nav1.5 was shown to potentiate VEGF-induced ERK1/2 activation by attenuating membrane depolarization, altering intracellular calcium kinetics and PKCα activity [173]. These findings identified Nav1.5 as a regulator of angiogenic function in endothelial cells and a potential novel strategy for controlling angiogenesis.

(c) *SCN5A*/Nav1.5 in (myo)fibroblasts

Fibroblasts play an important role in extracellular matrix formation and production of various paracrine and autocrine factors. Although they are non-excitable, fibroblasts can couple with other cell types including cardiomyocytes and affect their electrophysiological properties [174]. In human atrial fibroblast cultures, fast inward sodium current appeared *de novo* upon differentiation into myofibroblasts, which was predominantly TTX-resistant with properties similar to Nav1.5; in addition, *SCN5A* was the most abundant transcript identified [175]. Similarly, cultured fibroblasts isolated from right atrial tissue from patients with chronic atrial fibrillation demonstrated an increase in functional Nav1.5 channels upon differentiation into myofibroblasts [176]. A large window current was observed in myofibroblasts, which would be expected to lead to substantial sodium entry and consequently calcium influx [175]. While the latter could theoretically enhance myofibroblast proliferation, direct evidence is lacking; moreover, it remains to be investigated whether sodium channels promote fibroblasts differentiation into myofibroblasts and/or modulate myofibroblast secretion properties [177]. If so, it would open up potential novel avenues for therapeutic strategies to prevent fibrosis and ultimately arrhythmias.

11. Conclusion: insight gained and remaining questions

SCN5A/Nav1.5 (dys)function is clearly highly complex on multiple levels, not only in distinct cardiomyocyte subcellular microdomains, but also in other cell types within the heart (figure 3). While the consequences of *SCN5A* mutations are increasingly investigated in non-ventricular cardiomyocytes (e.g. atrial, conduction system), their potential impact on cells in the heart other than cardiomyocytes (e.g. SMCs, fibroblasts, neurons) has hardly been explored. Moreover, given the functional role of Nav1.5 in many non-cardiac tissues (figure 3), *SCN5A* mutations may result in extracardiac consequences which may or may not in turn exacerbate cardiac dysfunction and/or arrhythmias. Conversely, these non-cardiac clinical phenotypes may also provide potential new ways to diagnose and risk stratify patients with *SCN5A* mutations; for instance, one could also consider using biopsy material from extracardiac tissues (e.g. gastrointestinal) to study patient-specific impact of sodium channel dysfunction and potential therapeutic approaches. Regarding the latter, it should be taken into account that (pharmacological) interventions targeting Nav1.5 will impact not only the intended organ, but also other tissues including the heart. Hence, novel Nav1.5 modulators developed, for example, neurological and gastrointestinal disorders as well as cancer

may inadvertently lead to an increased risk for arrhythmias. Mechanistically, knowledge obtained on the effects of Nav1.5 (dys)function in non-excitable cells and tumour cells provides insight into the non-electrogenic role of the channel (including mechanosensitivity), which is increasingly recognized to contribute to the development of cardiomyopathy and other structural alterations secondary to *SCN5A* mutations. Regardless of the underlying mechanism, the latter will further predispose to arrhythmogenesis, contributing significantly to disease severity, progression and prognosis. Thus, therapeutic strategies aimed at preventing structural abnormalities secondary to *SCN5A* mutations may prove a promising approach for certain patients.

Taken together, research discussed in this review demonstrates that *SCN5A* channelopathies are more complex than previously appreciated, and raises many new questions, such as:

- Do *SCN5A* mutations modulate invasiveness of tumours, and as such are these patients at higher risk for metastasis and worse outcome when they get cancer?
- Do patients using sodium channel blocking drugs (e.g. for epilepsy) who subsequently develop cancer, have a less aggressive tumour with less extensive metastasis?
- Do *SCN5A* mutations lead to dysregulation of the autonomic nervous system, thereby potentially impacting on arrhythmogenesis?
- Are variants in *SCN5A* associated with worse outcome in MS?
- Are *SCN5A* mutations associated with an increased risk for schizophrenia?
- Can clinical parameters of, e.g. gastrointestinal or autonomic nervous system (dys)function be used for risk prediction in *SCN5A* mutation carriers?
- Do *SCN5A* mutations impact on vascular SMC function, thereby potentially increasing the risk for hypertension and/or (subclinical) ischaemia?
- Do *SCN5A* mutations affect (myo)fibroblast (dys)function, potentially inducing cardiac fibrosis?
- Do *SCN5A* mutations affect macrophage function and/or innate immune response, leading to inflammation and/or inappropriate auto-immunity?

Clearly, *SCN5A* channelopathies need to be approached in a more holistic, multidisciplinary manner, with collaboration between different specialists to improve patient management. Similarly, basic and translational research into *SCN5A* mutations and Nav1.5 dysfunction should address the various roles of this channel in different cell types and tissues using appropriate human and animal models. Ultimately, a multidisciplinary approach combining basic, translational, genetic and clinical studies should lead to improved diagnosis, risk stratification, treatment and outcome in patients with *SCN5A* channelopathy.

Data accessibility. This article has no additional data.

Conflict of interest declaration. I declare I have no competing interests.

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