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Cardiac sodium channel mutations: why so many phenotypes?

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Abstract

Mutations of the cardiac sodium channel ($\text{Na}_v1.5$) can induce gain or loss of channel function. Gain-of-function mutations can cause long QT syndrome type 3 and possibly atrial fibrillation, whereas loss-of-function mutations are associated with a variety of phenotypes, such as Brugada syndrome, cardiac conduction disease, sick sinus syndrome, and possibly dilated cardiomyopathy. The phenotypes produced by $\text{Na}_v1.5$ mutations vary according to the direct effect of the mutation on channel biophysics, but also with age, sex, body temperature, and between regions of the heart. This phenotypic variability makes genotype–phenotype correlations difficult. In this Perspectives article, we propose that phenotypic variability not ascribed to mutation-dependent changes in channel function might be the result of additional modifiers of channel behaviour, such as other genetic variation and alterations in transcription, RNA processing, translation, post-translational modifications, and protein degradation. Consideration of these modifiers might help to improve genotype–phenotype correlations and lead to new therapeutic strategies.

Introduction

A functional heart pumps blood throughout the body by regular, rhythmic contractions, which are controlled by electrical impulses usually initiated at specialized pacemaker sites and conducted throughout the heart, in part, by a myocardial conduction system. Under usual circumstances, the sinoatrial node, also known as the pacemaker, generates the initial depolarization that stimulates the atrial muscle to contract. The signal then travels to the atrioventricular node and conducts through the bundle of His and bundle branches to the Purkinje fibres. These fibres articulate with the subendocardial muscle, ensuring ventricular contraction from the apex to the base of the heart. This sequence of electrical activity can be monitored on the electrocardiogram (ECG).

The importance of the cardiac sodium channel ($\text{Na}_v1.5$) for cardiac electrical stability is highlighted by lethal arrhythmias that arise as the result of inherited genetic defects in the $\text{Na}_v1.5$ channel. Alterations in cardiac $\text{Na}_v1.5$ have also been implicated in arrhythmic risk

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Competing interests

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associated with acquired heart disease.^{1,2} The cardiac Na_v1.5 is responsible for the fast inward Na⁺ current (I_{Na}) that initiates the depolarizing phase 0 of the cardiac action potential (Figure 1), except in the sinoatrial and atrioventricular nodes. In addition to intercellular communication via gap junctions, Na_v1.5 is a major determinant of impulse conduction velocity in cardiac tissues.³

The biophysical properties of the sodium channel are complex. The α subunit of Na_v1.5, which is encoded by *SCN5A*, forms the ion-conducting pore and changes its structural conformation on a timescale of milliseconds in response to alterations in membrane potential. This process is termed voltage-dependent gating.⁴ The α subunit is composed of an intracellular N-terminus and C-terminus, and four homologous domains (I–IV) that form a pore to conduct Na⁺ ions across the cell membrane.⁵ The four domains are attached to one another by cytoplasmic linker sequences with important residues for channel gating and post-translational modifications, such as phosphorylation. Each domain contains six transmembrane segments (S1–S6). The four positively charged S4 segments constitute the voltage sensor, responsible for increased channel permeability during membrane depolarization.⁶ The S5 and S6 segments of all four domains form the ion-conducting pore, and the interconnecting P-loops make up the channel's selectivity filter.^{7,8} The α subunit interacts with accessory proteins, including β subunits and other proteins, to form a macromolecular complex that modulates channel expression and function (Figure 2).⁹

Mutations in *SCN5A* produce a variety of clinical phenotypes. Some portion of phenotypic variability is the result of the direct effects of the mutations on the Na_v1.5 biophysical properties. These effects are often divided into gain or loss of channel function (that is, increased or decreased I_{Na} , respectively). In gain-of-function mutations, an increase in the persistent late I_{Na} (I_{NaL}) during the action potential plateau, rather than in peak I_{Na} , is generally thought to be responsible for the phenotype. Gain-of-function mutations in *SCN5A* can result in long-QT syndrome type 3 (Figure 3). Conversely, loss-of-function mutations in *SCN5A* can lead to a decrease in peak I_{Na} , resulting in phenotypes including Brugada syndrome (BrS), sick sinus syndrome, cardiac conduction diseases, and possibly dilated cardiomyopathy. Some *SCN5A* mutations have also been shown to cause mixed phenotypes (overlap syndromes) or been linked with familial lone atrial fibrillation. Occasionally, mutations can cause both an increase in I_{NaL} and a simultaneous reduction in peak I_{Na} , thereby having both gain-of-function and loss-of-function properties.

The effects of mutations and their contribution to phenotypic variability have been extensively studied and reviewed previously,^{10–14} and so are not discussed further in this article. However, different *SCN5A* mutations can generate different clinical syndromes. These syndromes can manifest in a particular chamber or region of the heart. Additionally, the phenotype of a particular *SCN5A* mutation can show a variable penetrance between individuals, and can vary within an individual as a result of factors such as age, time of day, and body temperature. The source of this phenotypic variation is uncertain, and this degree of variability makes genotype–phenotype correlations difficult. Furthermore, the lack of correlation complicates medical decision-making in patients with known mutations.

In this Perspectives article, we propose that phenotypic variability not ascribed to mutation-dependent changes in channel biophysics might be the result of additional modifiers of channel behaviour. Established modifiers include additional genetic variants in the *SCN5A* gene. Other potential modifiers include alterations in the expression or function of the mutated channel (intrinsic modifiers), or changes in the expression of other channels or genes that can modify the overall electrical effect of an *SCN5A* mutation (extrinsic modifiers). Consideration of these modifiers might help to improve genotype–phenotype correlations and lead to new therapeutic strategies.

Phenotypic variability

The clinical phenotypic penetrance and manifestation of an *SCN5A* mutation can vary with sex, the presence of genetic modifiers, circadian rhythm, and ageing (Figure 4). For example, in patients with long QT syndrome type 3, men tend to have a longer corrected QT interval than women (Figure 4a).¹⁵ Furthermore, BrS affects far more men than women.^{16–18} Sex can influence the clinical phenotype of an individual mutation. For example, within a single family, four male members carrying a loss-of-function mutation in *SCN5A* (G1406R) had BrS, whereas seven other family members (six of whom were women) carrying the same mutation had cardiac conduction disease.¹⁷ The mechanism of this sex-dependent effect is still uncertain. Sex hormones, such as 5-dihydrotestosterone, oestrogen, and progesterone, might cause differences in QT intervals and gene expression of cardiac ion channels.¹⁹ For female patients, hormonal changes owing to menses and pregnancy might induce QT prolongation and increase susceptibility to arrhythmias.²⁰

The phenotype of *SCN5A* mutations can be age-dependent. For example, despite carrying the mutation since birth, patients with BrS do not typically develop an arrhythmic phenotype until aged approximately 34–53 years.²¹ Interestingly, the male predominance of an arrhythmic phenotype is not observed in children with BrS.²² Age-dependent hormonal changes have been proposed to explain the increased risk of spontaneous arrhythmias in men after puberty.^{22,23} Moreover, within an individual patient with an *SCN5A* mutation associated with BrS, phenotypic variability exists in the ECG manifestations of the disease as a function of time. A patient with BrS can show circadian changes in ECG patterns and susceptibility to lethal ventricular fibrillation (Figure 4c), and ECG manifestations can worsen with fever.²⁴ Furthermore, cardiac conduction diseases can be progressive and worsen with age (Figure 4d).²⁵

Intrinsic modifiers

The variability of phenotypes associated with an *SCN5A* mutation can result from another genetic variant that coexists within gene. For example, two BrS-associated *SCN5A* variants T1620M and R1232W are known to cause minor changes in Na_v1.5 channel properties when they exist independently.²⁶ When they coexist, however, the sodium channel T1620M/R1232W double mutant produces no current.²⁷ Individuals carrying either of the heterozygous loss-of-function mutations W156X or R225W do not have a clinical phenotype.²⁸ Compound heterozygosity for W156X and R225W mutations, however, leads to severe conduction disease, dilated cardiomyopathy, and death at a young age.²⁸ These

examples demonstrate that the coexistence of a genetic variant within the *SCN5A* gene can affect the severity of the phenotype associated with another *SCN5A* mutation. Additionally, the *SCN5A* H558R polymorphism has been found to be a disease-modifying genetic variant that can rescue gain-of-function²⁹ or loss-of-function³⁰ *SCN5A* mutations (Figure 4b), providing a potential mechanism to account for the variable penetrance that is often observed. Taken together, the presence of genetic modifiers within *SCN5A* might contribute to the phenotypic variability, even between family members who share the same mutation.

Any stage in the lifecycle of the cardiac Na_v1.5 channel is a potential modification point to amplify, localize, or minimize a mutation-mediated phenotype. The lifecycle of cardiac Na_v1.5 includes gene transcription, post-transcription RNA processing, translation (protein synthesis and assembly), post-translational trafficking and modifications, and protein degradation (Figure 5).^{31,32} Modifiers such as transcription factors, microRNAs, and kinases regulate these processes and result in functional changes of cardiac Na_v1.5. These modifiers do not have established roles in modifying inherited Na_v1.5 channelopathies, but they can vary with age, sex, clinical state, and between regions of the heart. Conceivably, therefore, variability in these modifiers might contribute to the overall phenotypic variability observed. Moreover, these modifiers are candidates to explain regional localization of Na_v1.5 mutation effects.

Transcriptional modifiers

Transcriptional regulation—Transcriptional dysregulation of *SCN5A* (Figure 5) can lead to either upregulation or downregulation of Na_v1.5 membrane expression and macroscopic I_{Na} . This change in sodium current can enhance or mask the phenotype of a mutation. Moreover, regional differences in transcriptional regulation might explain how various areas of the heart and conduction system are most affected.

Some transcriptional factors have essential roles in signalling normal *SCN5A* expression, dysregulation of which might disrupt *SCN5A* expression and cause phenotypic variability. T-box transcription factor TBX5 (TBX5) is essential for Na_v1.5 expression in the ventricular conduction system.³³ Removal of TBX5 causes a dramatic reduction in membrane expression of both Na_v1.5 and gap junction $\alpha 5$ protein (connexin-40), leading to conduction velocity slowing, cardiac arrhythmias, and increased mortality in animal models.³³ Alterations in TBX5 expression might modulate I_{Na} in the conduction system and contribute to conduction-specific phenotypes. *ARNTL*, encoding a core molecular clock transcription factor, also has an important role in *SCN5A* expression and cardiac arrhythmia susceptibility. Cardiomyocyte-specific deletion of *Arntl* in mice causes slowed heart rate, prolonged electrocardiographic RR and QRS intervals, and increased episodes of arrhythmia as a result of loss of *Scn5a* expression.³⁴ Alterations in *Arntl* might help to explain diurnal variations in arrhythmic risk in the presence of a particular mutation.

The *SCN5A* promoter region contains consensus binding sites for several transcription factors, such as nuclear factor NF- κ B (NF- κ B) and forkhead box protein O1 (FOXO1). When activated by angiotensin II or oxidative stress, NF- κ B and FOXO1 are upregulated and induce a significant decrease in Na_v1.5 expression and I_{Na} .^{35,36} Interestingly, transforming growth factor β 1 can counteract the effect of FOXO1 and increase the *SCN5A*

mRNA level and I_{Na} .³⁷ These data suggest that the phenotypic penetrance of a mutation might vary as a result of changes in these transcription factors. Additionally, single nucleotide polymorphisms in the promoter region of *SCN5A* have been shown to modulate the transcriptional activity of the gene,³⁸ and to affect the phenotypic severity in heterozygous carriers of an *SCN5A* loss-of-function mutation (1936delC).³⁹ These data suggest that genetic variants in the *SCN5A* promoter region can contribute to the phenotypic variability of *SCN5A* mutations through regulating *SCN5A* transcription.

mRNA processing and regulation—The phenotype of $Na_v1.5$ mutations can also be modified at the stage of mRNA processing. Our group has shown that angiotensin II and hypoxia can regulate *SCN5A* mRNA splicing and generate truncated, nonfunctional sodium channels in human heart failure.² These abnormally spliced, truncated channels also show a dominant-negative effect on the full-length *SCN5A* mRNA transcript by activating eukaryotic translation initiation factor 2 α kinase 3 (PERK; Figure 5).⁴⁰ Angiotensin II can be differentially regulated in local regions of the heart. In rats with type 1 diabetes mellitus, for example, the angiotensin II level is elevated in ventricular cells, but not in atrial cells.⁴¹ Combining local regulation with induction of abnormal mRNA processing might contribute to a phenotype that varies according to clinical state.

MicroRNAs are endogenous, noncoding, small ribonucleic acids that are emerging as important regulators of gene expression by either restricting translation or inducing degradation of the target mRNA. Altered expression (mainly upregulation) of microRNAs has been widely reported to reduce the expression of cardiac ion channels in studies of cardiovascular diseases.^{42–44} In heart failure, upregulated microRNA-125a/b was associated with suppression of $Na_v1.5$ protein expression and a reduction in I_{Na} .⁴⁴ A stress-inducible microRNA, microRNA-195, which is upregulated in cardiac hypertrophy and heart failure,⁴⁵ can downregulate *SCN5A*, leading to a reduction in I_{Na} .⁴³ In myocardial infarction, upregulation of the let-7f microRNA and microRNA-378 also causes a reduction in I_{Na} .⁴⁴ Therefore, changes in microRNAs over time might contribute to phenotypic variability.

Other microRNAs might have indirect effects on the $Na_v1.5$ channel. For example, microRNA-199a is downregulated after induction of ischaemia, leading to an upregulation of its target, hypoxia-inducible factor 1 α . This factor can activate RNA-binding protein 25,⁴⁶ and increase abnormal *SCN5A* mRNA splicing to generate truncated, nonfunctional sodium channels.^{2,40} In human atrial fibrillation and ventricular arrhythmia, microRNA-1 is markedly decreased, which can induce upregulation of the $K_{ir2.1}$ potassium channel.⁴⁷ This channel conducts an inward potassium current (I_{K1}) that controls the resting membrane potential (Figure 1). An increase in I_{K1} can lead to membrane hyperpolarization, which can either amplify the reduction in I_{Na} induced by an *SCN5A* loss-of-function mutation, or minimize the increase in I_{NaL} induced by an *SCN5A* gain-of-function mutation. In a canine tachypaced model of atrial fibrillation, the level of microRNA-328 was increased, and led to a reduction in the voltage-gated L-type calcium channel current and a shortened atrial action-potential duration.⁴⁸ These changes might combine with those of $Na_v1.5$ loss-of-function mutations to worsen arrhythmic risk.

Translational modifiers

At the translational level, many modifiers regulate Na_v1.5 protein synthesis, folding, trafficking, and assembly with accessory subunits and interacting proteins. For example, activated mitogen-activated protein kinases (MAPKs), such as p38-MAPK and MAPK8,^{49,50} can affect translation of the Na_v1.5 channel and gap junction α 1 protein (connexin-43) and contribute to arrhythmia.^{51–54}

Trafficking to cell membrane—After *SCN5A* is translated to protein and assembled in the endoplasmic reticulum, the channel is transited to the Golgi complex, where Na_v1.5 channel trafficking begins. A Golgi vesicle containing the Na_v1.5 channel is then transported to the plasma membrane area. Modifiers such as cAMP-dependent protein kinase (also known as protein kinase A; PKA) and protein kinase C (PKC) regulate channel trafficking to the plasma membrane. For instance, PKC activation can decrease Na_v1.5 channel trafficking, whereas PKA can have the opposite effect.^{55,56} Therefore, a patient's clinical state might affect the penetrance of a mutation.

Caveolae (small invaginations of the cell membrane) are potential Na_v1.5 modifiers, because colocalization of the Na_v1.5 protein and caveolin-3 is observed in ventricular tissues.⁵⁷ Caveolae constitute a reservoir of I_{Na} that can be modulated by opening or closing of the caveolar neck.⁵⁸ Caveolae also have a high density of signalling molecules, such as G proteins, PKA, PKC, and Src family tyrosine kinases.^{59–61} A complex formed between caveolin-3, G α protein, and PKA in caveolae modulates Na_v1.5 function.^{61,62} PKA and PKC regulate Na_v1.5 gating properties by phosphorylation.⁶³ A caveolin-3 mutation results in a three-fold increase in I_{NaL} , which might enhance the penetrance of long QT syndrome type 3 induced by Na_v1.5 gain-of-function mutations.⁶⁴ Any changes in these molecules will affect Na_v1.5 membrane availability and alter the phenotype of Na_v1.5 mutations.

Regulation of the channel complex—The Na_v1.5 α subunit interacts with accessory proteins (β subunits and interacting proteins, reviewed previously⁶⁵) to form a macromolecular complex (Figure 2). Mutations of these accessory proteins have been reported to cause BrS. For example, patients with BrS and mutations in the sodium channel β 1 and β 3 subunits show impaired Na_v1.5 channel function.^{12,66,67} The expression level of the β 4 subunit is reported to affect the severity of cardiac conduction disease in mice by modulating Na_v1.5 channel activation.⁶⁸

Na_v1.5 interacting proteins, such as ankyrin, Ran guanine nucleotide release factor (MOG1), and syntrophin, interact with the α and β subunits of the sodium channel. Mutations and misregulation of these proteins can disturb Na_v1.5 membrane expression and function. For example, a mutation in ankyrin-2 causes long QT syndrome type 4, resulting from disruption of ankyrin-2 interactions with Na_v1.5, the sodium/calcium exchanger, and inositol 1,4,5-trisphosphate receptors.⁶⁹ Mutations in the MOG1 gene (*RANGRF*) are found in patients with BrS (E83D)⁷⁰ and atrial fibrillation (E61X).⁷¹ The A257G syntrophin mutation induces long QT syndrome type 3 owing to a gain-of-function modulation of Na_v1.5 gating properties.⁷² Therefore, differential regulation of the macromolecular complex is another potential modifier of the phenotype induced by a Na_v1.5 mutation.

Post-translational modifiers

PKA and PKC—The best-defined post-translational modifiers of cardiac $\text{Na}_v1.5$ are PKA and PKC, which can modify channel expression and gating properties. Combined effects of these modifiers with the baseline genetic defect might explain phenotypes that vary with time, clinical state, and between regions of the heart.

Protein kinases regulate $\text{Na}_v1.5$ in fast and complex ways. For example, PKA and PKC regulate $\text{Na}_v1.5$ by affecting channel conductance, trafficking, and membrane expression.^{1,55,56,73} PKCs are a family of serine/threonine protein kinases with at least 15 isoforms in humans, with the PKC α , δ , and ϵ isoforms being most-intensively investigated in cardiomyocytes.⁷⁴ PKC decreases the cardiac I_{Na} by channel redistribution away from plasma membrane.⁵⁶ Additionally, PKC δ reduces I_{Na} by inducing mitochondrial overproduction of reactive oxygen species (ROS) and decreasing the single-channel conductance.^{1,63,75,76} These effects occur within minutes, without altering *SCN5A* mRNA abundance or channel membrane expression, suggesting that they are independent of effects on channel trafficking. Therefore, PKC activation generally reduces I_{Na} and might exacerbate the phenotype of *SCN5A* loss-of-function mutations or mitigate the phenotype of *SCN5A* gain-of-function mutations. PKC ϵ has not been found to regulate $\text{Na}_v1.5$ directly, but activation of PKC ϵ has been reported to have a beneficial antiarrhythmic effect in a PKC ϵ agonist transgenic mouse model of ischaemia–reperfusion injury.⁷⁷ This finding indicates that the state of PKC ϵ activation might modulate the phenotypic variance of $\text{Na}_v1.5$ mutations.

PKA is a tetrameric serine/threonine kinase with broad specificity that is known to upregulate $\text{Na}_v1.5$ through changes in channel trafficking⁵⁵ and conductance.⁶³ Important PKA phosphorylation sites on human cardiac $\text{Na}_v1.5$ at Ser525, Ser528, Arg533, Arg534, and Arg535 have been identified.^{55,78,79} Arg533–535 are important for PKA-dependent increases in I_{Na} .⁶³ PKA can also upregulate $\text{Na}_v1.5$ through minimizing mitochondrial ROS production.^{1,75} Furthermore, PKA activation of gap junction $\alpha 1$ protein (connexin-43) stimulates gap junctional intercellular communication and decreases the electrical distance over which $\text{Na}_v1.5$ mutations have their effects.^{80–82}

ROS—Major sources of cardiac ROS include the mitochondrial electron transport chain, uncoupled nitric oxide synthase, NAD(P)H oxidase, cyclo-oxygenase, and xanthine dehydrogenase/oxidase. Enzymes such as superoxide dismutase, glutathione per-oxidase, and catalase reduce ROS levels. ROS elevation is a common feature shared by various cardiac diseases.^{83,84} Increasing evidence suggests that elevated levels of ROS affect the cardiac ion channels (such as $\text{Na}_v1.5$, $\text{K}_v1.4$, $\text{K}_v4.2$, $\text{K}_v4.3$, and $\text{Ca}_v1.2$), Ca^{2+} -handling proteins, and gap-junction proteins.⁸⁵ ROS can downregulate $\text{Na}_v1.5$ at the transcriptional level by reducing *SCN5A* expression,^{35,40} and at the post-translational level by affecting channel phosphorylation and conductance.⁶³ For example, mitochondrial ROS can modify $\text{Na}_v1.5$ directly via an unknown mechanism that decreases I_{Na} at the post-translational level without changing channel membrane expression.⁶³ Mitochondrial ROS can also activate PKC,^{1,75} which can further downregulate channel activity by phosphorylation⁶³ or impairment of channel trafficking.⁸⁶

Alterations in metabolism—Heart failure and cardiac injury from many causes are associated with altered metabolism and downregulation of $\text{Na}_v1.5$.^{87–90} The level of NADH is known to oscillate during myocardial ischaemia, and mitochondrial injury is associated with increased NADH and ROS levels.^{91,92} An elevated level of NADH results in PKC activation, mitochondrial ROS overproduction, and decreased I_{Na} .^{1,75} Many such alterations might be localized to ischaemic regions of myocardium. By activating PKA, reducing mitochondrial ROS production, and enhancing I_{Na} , NAD^+ antagonizes the reduction in I_{Na} associated with a rise in the level of NADH.^{1,75} In an *ex vivo* study, NADH perfusion led to ventricular tachycardia induced by programmed electrical stimulation in wide-type mouse hearts, whereas NAD^+ perfusion diminished the incidence of ventricular tachycardia in hearts from *SCN5A*^{+/-} mice.¹ Therefore, any alteration in cardiac metabolism might alter the phenotype of $\text{Na}_v1.5$ mutations.

Degradation pathways—The last step of the $\text{Na}_v1.5$ channel lifecycle is degradation. A balance between synthesis and degradation of the channel determines the channel population available to generate currents. Ubiquitination of membrane proteins by ubiquitin-protein ligases is a common signal for protein internalization and degradation. E3 ubiquitin-protein ligase NEDD4 (NEDD4) is a ubiquitin protein ligase that regulates the degradation of cardiac $\text{Na}_v1.5$, $\text{K}_v\text{LQT1}$, and $\text{Ca}_v1.2$ channels. In *Xenopus* oocytes, Nedd4 significantly reduces the membrane expression of these channels, and attenuates ion currents by 40–75%.^{51,93–96} An inactive Nedd4 mutant can increase the $\text{Na}_v1.5$ density on the membrane and elevate I_{Na} .⁹³ NEDD4 also regulates the degradation of neuronal sodium channels ($\text{Na}_v1.2$, $\text{Na}_v1.3$, $\text{Na}_v1.7$, and $\text{Na}_v1.8$), which are expressed in cardiomyocytes and affect cardiac function (as discussed below).^{95–97} NEDD4 can be regulated by PKA, MAPKs, serine/threonine-protein kinase Sgk, and 14-3-3 proteins.^{51–53,98,99} Therefore, any changes in NEDD4 and its modifiers might affect the membrane expression of cardiac ion channels, which will give rise to various phenotypes when combined with $\text{Na}_v1.5$ mutations.

Extrinsic modifiers

Other sodium channel isoforms

In addition to cardiac $\text{Na}_v1.5$, the neuronal and skeletal isoforms of voltage-gated sodium channels ($\text{Na}_v1.1$ to $\text{Na}_v1.8$) are also expressed in the heart. In adult mouse hearts, $\text{Na}_v1.5$ is responsible for 92% of I_{Na} , whereas the other isoforms account for 8%.¹⁰⁰ $\text{Na}_v1.1$ and $\text{Na}_v1.6$ participate in pacemaking in the sinoatrial node,¹⁰¹ and in excitation–contraction coupling.¹⁰² $\text{Na}_v1.6$ has also been shown to act as a depolarizing reserve to ensure excitation.¹⁰² Mutations in $\text{Na}_v1.8$, encoded by *SCN10A*, have been associated with prolonged cardiac conduction and an increased risk of heart block.¹⁰³ $\text{Na}_v1.8$ is mainly expressed in intracardiac neurons of the heart and has an important role in regulating the frequency of action-potential firing in mice.¹⁰⁴ This channel also contributes to I_{NaL} , and its inhibition might be antiarrhythmic.¹⁰⁵ An association between common variants in *SCN5A*, *SCN10A*, and a transcriptional regulator gene *HEY2* was reported in a genome-wide association study of patients with BrS.¹⁰⁶ The cumulative effect of the three loci significantly decreased cardiac conduction velocity and increased susceptibility to BrS.

Differential regulation of these neuronal and skeletal isoforms of sodium channels might modulate the phenotype of any $\text{Na}_v1.5$ mutation.

Other cardiac ion channels

Mutations in genes encoding channels other than $\text{Na}_v1.5$ are also associated with BrS and long QT syndrome type 3, which suggests that many different channels can be involved in generating one clinical phenotype. This idea implies that changes in these other ion channels can affect the phenotype of any $\text{Na}_v1.5$ mutation. For example, the *SCN5A* mutation D1275N has been shown to cause atrial standstill if this mutation is combined with polymorphisms in the atrial-specific gap junction $\alpha 5$ protein (connexin-40).¹⁰⁷ The potassium current I_{to} (conducted by $\text{K}_v4.2$ and $\text{K}_v4.3$) and I_{K1} (conducted by $\text{K}_{ir2.1}$) have been shown to modify I_{Na} and affect the action-potential duration and conduction velocity. I_{to} counteracts the depolarizing currents during the early phase of the action-potential plateau, resulting in an action-potential notch (phase 1) and a ‘spike-and-dome’ morphology (Figure 1). In a canine heart model, increased I_{to} at the early phase of action potential was found to cause conduction slowing.¹⁰⁸ When combined with I_{Na} reduction, I_{to} that is larger in subepicardial than in subendocardial myocytes can lead to loss of the action-potential dome and early completion of repolarization in the right ventricular subepicardium, but not the subendocardium. This situation can result in ST-segment elevation being evident on the ECG in patients with BrS. Therefore, alterations or mutations of $\text{K}_v4.3$ and its accessory proteins could amplify early after depolarizations or mitigate early repolarization in BrS.

I_{K1} controls the resting membrane potential, which determines voltage-gated $\text{Na}_v1.5$ availability. I_{K1} – I_{Na} interactions have been shown to involve reciprocal modulation of their respective channel expressions within a macromolecular complex.¹⁰⁹ An increase in I_{K1} expression can lead to membrane hyperpolarization, which can either amplify a reduction in I_{Na} induced by *SCN5A* loss-of-function mutations, or minimize an increase in I_{NaL} induced by *SCN5A* gain-of-function mutations.

Genetic and genomic background

Genetic background can contribute to the variable phenotypes observed with *SCN5A* mutations. A study in families with BrS carrying *SCN5A* mutations suggested that the presence of the Brugada phenotype is determined not only by *SCN5A* mutation, but also by the genetic background of an individual.¹¹⁰ The *SCN5A*^{1798insD/+} mutation has been found to generate a more-severe phenotype of conduction slowing in mice with a 129P2 genetic background than in those with a FVB/N background.⁶⁸ A genome-wide association study in patients with BrS showed that polymorphisms in *SCN10A* and *HEY2* can have a large effect on the phenotype of BrS.¹⁰⁶ Therefore, multiple genetic mutations in unrelated genes can act to lower I_{Na} and cause BrS, which highlights the importance of considering genetic and genomic modifiers when undertaking genotype–phenotype correlations.^{66,67,111,112}

Conclusions

Mutations in the cardiac $\text{Na}_v1.5$ can result in various clinical syndromes. Some of this phenotypic variability can be explained by the direct effect of the mutation on channel

properties and biophysics. Conversely, manifestation of the mutation in a particular region or chamber of the heart, circadian variation, age-dependent arrhythmic episodes, incomplete penetrance in families, and variations according to clinical state within an affected individual are difficult to explain. We propose that this variability might be the result of secondary modifiers of the $\text{Na}_v1.5$ channel. We have divided these modifiers into those within the *SCN5A* gene, those intrinsic to the lifecycle of $\text{Na}_v1.5$, and those extrinsic to $\text{Na}_v1.5$, which can modify overall electrical function. However, much of the evidence for modifiers of $\text{Na}_v1.5$ expression and function has been derived from the literature on heart failure. Further experimental evidence will be required to support and generalize this ‘second-hit’ hypothesis of phenotypic variability to the field of arrhythmia syndromes associated with *SCN5A* mutations.

$\text{Na}_v1.5$ channel modifiers might conceivably alleviate or aggravate the phenotype of an *SCN5A* mutation, depending on their effect on the sodium current or the overall balance of currents. Localized modifier changes might explain regional expression of mutation effects. Changes in modifiers might also explain why some conduction disease phenotypes progress and others do not. Variations in modifiers could explain why ECG manifestations and arrhythmic risk varies with time of day and with age. These considerations might also explain why less phenotypic variability exists in gain-of-function mutations than in loss-of-function mutations, because fewer modifiers exist that affect only inactivation than affect overall channel current. Nevertheless, the net effect of a gain-of-function mutation can be modulated by the total number of available channels, which might explain the variable penetrance of a phenotype with time or between regions of the heart. In summary, considering these modifiers might improve genotype–phenotype correlation and risk-prediction algorithms. Moreover, targeting modifiers might prove to be an effective strategy to prevent diseases associated with $\text{Na}_v1.5$ mutations.

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Figure 1.

A schematic representation of the cardiac action potential with the major contributing cardiac ion channel currents. Reprinted from Liu, M., Brahmanandam, V. M. & Dudley, S. C. Jr in *Biophysics of the Failing Heart: Physics and Biology of Heart Muscle*, 1st edn Ch. 4 Biophysics of Membrane Currents in Heart Failure (eds Solaro, R. J. & Tardif, J. C.) 63–90 (Springer, 2013), with kind permission from Springer Science and Business Media.

Figure 2.

The Na_v1.5 channel is part of a macromolecular complex. The channel α subunit interacts with multiple cellular proteins, including accessory proteins such as β subunits, ankyrin, CAV3, MOG1, syntrophin, and cytoskeleton at the cell membrane, forming a macromolecular complex. Na_v1.5 channel activity can be modified by the altered expression or function of the components of the macromolecular protein complex. Abbreviations: CAV3, caveolin-3; MOG1, Ran guanine nucleotide release factor.

Figure 3.

Mutations in *SCN5A* can produce various clinical phenotypes. *SCN5A* gain-of-function mutations can result in increased late I_{Na} , leading to long QT syndrome type 3. *SCN5A* loss-of-function mutations can lead to decreased peak I_{Na} , which is associated with Brugada syndrome, sick sinus syndrome, cardiac conduction diseases, and possibly dilated cardiomyopathy. Moreover, *SCN5A* mutations that cause both a gain in late I_{Na} and a loss of peak I_{Na} can be associated with a mixed phenotype or overlap syndromes (for example, Brugada syndrome and long QT syndrome type 3). Similarly, both gain-of-function and loss-of-function mutations have been associated with familial lone atrial fibrillation.

Figure 4.

Examples of phenotypic variability in patients carrying an *SCN5A* mutation. **a** | Sex can affect the phenotype of long QT syndrome type 3. In these patients, men tend to have a longer QTc interval than women.¹⁵ **b** | Genetic variants within or outside the gene can alter the phenotype of a *SCN5A* mutation. For example, the *SCN5A* H558R polymorphism has been shown to rescue the phenotype of P2006A and R282H mutations in *SCN5A*.^{29,30} **c** | Circadian changes in electrocardiogram patterns and the susceptibility to lethal ventricular fibrillation have been shown in Brugada syndrome. An electrocardiogram that is typical of Brugada syndrome (with ST-segment elevation in the precordial leads) is more-frequently observed in the midnight-to-early-morning period during sleep than during the day.¹¹³ **d** | As a patient ages, *SCN5A* mutations have been shown to cause progressive cardiac conduction defects, including deterioration in ventricular conduction. These serial electrocardiograms show progressive widening of the QRS complex with ageing.²⁵ Abbreviation: QTc, corrected QT interval. Panel c reprinted from Mizumaki, K. *et al.* Vagal activity modulates spontaneous augmentation of ST elevation in the daily life of patients with Brugada syndrome. *J. Cardiovasc. Electrophysiol.* **15** (6), 667–673 (2004), with permission from John Wiley and Sons. Panel d was published in *J. Am. Coll. Cardiol.* **41** (4), Probst, V. *et al.* Haploinsufficiency in combination with aging causes *SCN5A*-linked hereditary Lenègre disease. 643–652 © Elsevier (2003).

Figure 5.

The lifecycle of cardiac $\text{Na}_v1.5$ and potential phenotypic modifiers. The lifecycle starts with *SCN5A* gene transcription in the nucleus, which can be regulated by transcription factors, such as FOXO1, NF- κ B, and TBX5. The next steps are protein translation and assembly in the endoplasmic reticulum and Golgi apparatus. Appropriately folded $\text{Na}_v1.5$ protein is trafficked to the cell membrane, whereas misfolded protein (as a result of mutations and splicing variants) can activate the PERK pathway of the unfolded protein response to downregulate the *SCN5A* mRNA level. microRNAs also regulate the *SCN5A* mRNA level. Channel trafficking can be modulated by PKA, PKC, and caveolae. After being expressed on the cell membrane, NaChIPs join the α and β subunits to form a macromolecular complex, which can be regulated by PKA, PKC, oxidative stress (ROS), and metabolic states (NADH and NAD⁺). Eventually, the channel will go through ubiquitin-mediated degradation, which can be regulated by NEDD4. Abbreviations: CAV3, caveolin-3; FOXO1, forkhead box protein O1; NaChIP, Na⁺-channel-interacting protein; NEDD4, E3 ubiquitin-protein ligase NEDD4; NF- κ B, nuclear factor NF- κ B; PERK, eukaryotic translation initiation factor 2 α kinase 3; PKA, cAMP-dependent protein kinase (protein kinase A); PKC, protein kinase C; ROS, reactive oxygen species; TBX5, T-box transcription factor TBX5.