Phenotypical Manifestations of Mutations in the Genes Encoding Subunits of the Cardiac Sodium Channel

Arthur A.M. Wilde, Ramon Brugada

Abstract: Variations in the gene encoding for the major sodium channel (Na,1.5) in the heart, SCN5A, has been shown to cause a number of arrhythmia syndromes (with or without structural changes in the myocardium), including the long-QT syndrome (type 3), Brugada syndrome (progressive) cardiac conduction disease, sinus node dysfunction, atrial fibrillation, atrial standstill, and dilated cardiomyopathy. Because many of these diseases associate with sudden death in young individuals with a structurally normal heart, potentially causal SCN5A variants have also been identified in sudden infant or sudden unexplained death syndrome (SIDS/SUDS). Detailed descriptions of the clinical (patients) and basic electrophysiological phenotypes (heterologous expressed wild-type and mutant sodium channels and their interacting proteins), and on attempts to integrate the obtained knowledge, the past 15 years has witnessed an explosion of knowledge about these disease entities. (Circ Res. 2011;108:884-897.)

Key Words: sodium channel | arrhythmias | sudden cardiac death
β-subunits (β1 to β4), the α-subunit Na_1.5 also interacts with several regulatory proteins, of which, more recently, malfunction resulting from disease-causing variants in their encoding genes has been shown to underlie comparable phenotypes.

This article aims to review the existing knowledge of the basic and clinical aspects of sodium channel related diseases. It begins with a review of sodium channel function in normal and abnormal conditions that is followed by a description of the diverse clinical phenotypes.

The Cardiac Sodium Channel

The α-subunit of the cardiac isoform of the sodium channel is known as NaV1.5 and is encoded by the SCN5A gene. SCN5A maps to the short arm of chromosome 3 (3p21), contains 28 exons spanning ~80 kb, and encodes the α-subunit of the voltage-gated cardiac sodium channel (designated Na_1.5), conducting the cardiac sodium current I_{Na}. Sodium channels are expressed in several tissues and share a common structure. Hence, the cardiac sodium channel is a member of the voltage-dependent family of sodium channels, which consist of heteromeric assemblies of the pore-forming α-subunit. NaV1.5 contains 2016 residues and has an approximate molecular mass of 227 kDa. It consists of 4 homologous domains, known as DI to DIV, joined by so-called linkers. These 3 linkers, as well as the N terminus and the C terminus of the protein, are cytoplasmic. Each domain, DI to DIV, contains 6 transmembrane helices (namely S1 to S6) linked by intracellular or extracellular loops. S5 and S6 in each of the domains form the pore-lining helices (Figure 1).

Opening of the channel allows a rapid influx of positively charged Na ions (I_{Na}) that will depolarize within tenths of a millisecond the membrane potential of cardiac cells. As such,
sodium channel activity plays a central role in cardiac excitability and conduction of the cardiac impulse. The S4 segments in each domain, containing a positively charged amino acid at every third position, are held responsible for voltage-dependent activation. At the end of the action potential upstroke (ie, phase 0), most channels are inactivated and do not further allow passage of ions. Furthermore, this inactivation process is voltage-dependent. It is generally accepted that inactivation is mediated mainly by the inactivation gate (DIII to DIV linker), which blocks the inside of the channel shortly after it has been activated. Furthermore, the C-terminal cytoplasmic domain plays an important role in inactivation. Channels can only be reactivated after recovery from inactivation during phase 4. However, a small percentage of channels remain available to conduct and may reopen during the plateau phase of the action potential (phases 2 and 3). This fraction of channels, less than 1% of total available Na\(^{+}\) channels, contributes a small “late” Na\(^{+}\) current (\(I_{\text{NaL}}\)) to total membrane current. Under normal conditions, \(I_{\text{NaL}}\) is very small and has little impact on action potential morphology but can be very important in pathological conditions involving sodium channel mutations, among others (see below).

The cardiac Na\(^{+}\) channel is a multiprotein complex in which auxiliary proteins interact with the \(\alpha\)-subunit (Na\(_{\alpha},1.5\)) to regulate its gating, cellular localization, intracellular transport, and degradation. By performing screening experiments such as coimmunoprecipitation and yeast 2-hybrid, interactions with Na\(_{\alpha},1.5\) have been demonstrated for several proteins. In addition, mapping techniques have been used to discover the sites of interactions and the regulatory role of these auxiliary proteins have been studied with coexpression experiments in heterologous expression systems such as human embryonic kidney cells (HEK-293), Chinese hamster ovary cells (CHO), or *Xenopus* oocytes. Recently, genetic screening studies have linked mutations in genes encoding several of these subunits of the cardiac Na\(^{+}\) channel to phenotypes similar to those previously associated with mutations in SCN5A. Auxiliary proteins that interact with Na\(_{\alpha},1.5\) may be classified as enzymes, adapter proteins, and regulatory proteins. Enzymes may directly interact with Na\(_{\alpha},1.5\) and control its cellular localization by ubiquitilation and internalization (ubiquitin-protein ligases). Enzymes may also alter gating properties by phosphorylation (kinases) or dephosphorylation (phosphatase). Adapter proteins anchor Na\(_{\alpha},1.5\) to the cytoskeleton and play a role in the intracellular transport and targeting of Na\(_{\alpha},1.5\) to the sarcolemma. Regulatory proteins modulate gating properties of Na\(_{\alpha},1.5\) on binding.\(^{16}\)

As indicated mutations in SCN5A lead to various phenotypes. In addition, mutations in 7 genes that encode for auxiliary proteins of the cardiac Na\(^{+}\) channel have been linked to arrhythmic phenotypes previously associated with these phenotypes (Table 1). Four of these genes (SCN1B, SCN2B, SCN3B, and SCN4B) encode for proteins with a uniform molecular structure (\(\beta1, \beta2, \beta3\), and \(\beta4\), respectively; Figure 1). These so-called \(\beta\)-subunits of the cardiac Na\(^{+}\) channel contain an extracellular amino terminus, a single transmembrane segment, and an intracellular carboxyl terminus. All 4 \(\beta\)-subunits are expressed in the heart, and are particularly localized at the T-tubules/Z-lines and intercalated disks in cardiac myocytes.\(^{17}\) The \(\beta\)-subunits play critical roles in the regulation of sarcolemmal expression and gating of Na\(_{\alpha},1.5\), in the recruitment of cytoskeletal adapter proteins, enzymes, and signaling molecules to the channel and in the adhesion of Na\(_{\alpha},1.5\) to the cytoskeletal framework and extracellular matrix.

For many of the identified putative causal variants in both SCN5A, as well as in all its subunits (see below), one should realize that almost always sound segregation data, linking the genetic variant to the clinical phenotype lack. Other evidence that the identified variants are disease-causing is based on “weaker criteria” that include absence in control alleles, conservation throughout species and functional data. Indeed, in a study designed to identify the presence of rare variants in the 3 main genes associated with LQTS, including SCN5A, in

<table>
<thead>
<tr>
<th>Gene</th>
<th>Locus</th>
<th>Protein</th>
<th>Phenotype(s)</th>
<th>Effect of Mutation(s) on (I_{\text{Na}})</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCN1B</td>
<td>19q13.1</td>
<td>(\beta1)-subunit</td>
<td>Brugada syndrome, Cardiac conduction disease, Atrial fibrillation</td>
<td>Decrease in peak (I_{\text{Na}})</td>
<td>48, 74</td>
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<tr>
<td>SCN2B</td>
<td>11q23</td>
<td>(\beta2)-subunit</td>
<td>Atrial fibrillation</td>
<td>Decrease in peak (I_{\text{Na}})</td>
<td>48</td>
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<tr>
<td>SCN3B</td>
<td>11q23.3</td>
<td>(\beta3)-subunit</td>
<td>Brugada syndrome, Idiopathic ventricular fibrillation, Sudden infant death syndrome, Atrial fibrillation</td>
<td>Decrease in peak (I_{\text{Na}})</td>
<td>49, 79, 82</td>
</tr>
<tr>
<td>SCN4B</td>
<td>11q23.3</td>
<td>(\beta4)-subunit</td>
<td>Long QT syndrome type 10, Sudden infant death syndrome</td>
<td>Increase in persistent (I_{\text{Na}})</td>
<td>84, 85</td>
</tr>
<tr>
<td>GPD1L</td>
<td>3p22.3</td>
<td>Glycerol 3 phosphate dehydrogenase 1-like (GPD1L)</td>
<td>Brugada syndrome, Sudden infant death syndrome</td>
<td>Decrease in peak (I_{\text{Na}})</td>
<td>90, 93</td>
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<tr>
<td>CAV3</td>
<td>3p25</td>
<td>Caveolin-3</td>
<td>Long QT syndrome type 9, Sudden infant death syndrome</td>
<td>Increase in persistent (I_{\text{Na}})</td>
<td>96, 100</td>
</tr>
<tr>
<td>SNTA1</td>
<td>20q11.2</td>
<td>(\alpha)-1 syntrophin</td>
<td>Long QT syndrome type 12, Sudden infant death syndrome</td>
<td>Increase in (persistent) (I_{\text{Na}})</td>
<td>107, 108</td>
</tr>
</tbody>
</table>
control individuals, these variants were particularly identified in the intra domain linker and the C terminus of SCN5A. All of these variants were altered amino acids considered well-conserved among species. This has led to the notion that the pretest probability of pathogenicity of SCN5A variants identified in these regions to cause LQTS3 (see below) is far less than 50%. With this caveat in mind, one should examine the existing literature and be very cautious in calling a rare genetic variant a novel arrhythmia syndrome–susceptibility mutation. This holds in particular for variants identified in single patients only once. Clearly, detailed functional studies demonstrating clear aberration from normal behavior increase the likelihood that certain variants are disease-causing.

**Clinical Phenotypes Related to SCN5A Mutations**

**Brugada Syndrome**

BrS is a hereditary disease responsible for ventricular fibrillation and SCD in the young. The disease is characterized by the presence of right ventricular conduction abnormalities and coved-type ST-segment elevation in the anterior precordial leads (V1 to V3). The prevalence of BrS is estimated at approximately 3 to 5 in 10,000 people. Although the mean age of onset of events is approximately 40 years, SCD can affect individuals of any age, particularly men (75%). Of those, 20% to 50% have a family history of SCD. The disease exhibits an autosomal dominant pattern of transmission and variable penetrance. Most mutations occur in genes related to Na⁺ channels, although other channels may also be implicated in BrS. Between 20% and 25% of patients affected by BrS have mutations in the SCN5A gene. To date, there are more than 200 known SCN5A BrS-related mutations described. These mutations lead to a loss of Na⁺ channel function through several mechanisms, including trafficking defects, generation of truncated proteins, faster channel inactivation, shift of voltage dependence of steady-state activation toward more depolarized membrane potentials (Figure 2), or slower recovery from inactivation. Recently, it has been shown that in several large SCN5A-related BrS families (5 of 13 families with 4 or more phenotypically affected) affected individuals did not carry the familial disease-causing mutation. These observations could be explained by phenocopies but could also be explained by the presence of an anatomic substrate (an area with slow conduction in the RV outflow track) with a strong modifying role of SCN5A loss-of-function mutations. Apparently, in some individuals, the substrate is sufficient to provide the Brugada pattern spontaneously, ie, in the absence of an SCN5A mutation. In any case, these observations question a direct causal role of SCN5A mutations, and it stresses once more how careful one should be when investigating mutations.

Genotype–phenotype correlation studies in BrS have been limited because of the low prevalence of mutation carriers. A recently published study has proposed that the type of SCN5A mutation may be useful for risk stratification. In this study, a genotype-phenotype correlation was performed according to the type of SCN5A mutation (missense, truncated).

Patients and relatives with a truncated protein had a more severe phenotype and more severe conduction disorders. At this point in time, such data are too preliminary to use in clinical decision making.

**Lev-Lenègre Syndrome**

Lev-Lenègre syndrome is a progressive cardiac conduction disease (characterized by either prolongation of the P wave, PR or QRS segment duration, complete AV block, and bundle branch blocks) probably associated with gradually developed ventricular fibrosis of the conduction system. The first locus for the disease was reported in 1995, on chromosome 19q13.2-13.3. In 1999, the first mutations causing the disease were described in SCN5A. Mutations in SCN5A lead to a reduction in Na⁺ current, reducing the velocity of the impulse conduction.

**Long QT Syndrome**

LQTS is a cardiac channelopathy characterized by a QT interval prolongation, responsible for ventricular tachyarrhythmias with associated episodes of syncope and SCD. LQTS is one of the leading causes of SCD among young people. It can be congenital or acquired, generally in association with drugs and electrolyte imbalance (hypokalemia, hypocalcemia and hypomagnesemia). The clinical presentation can be variable, ranging from asymptomatic patients to episodes of syncope and SCD caused by ventricular tachyarrhythmias (torsades de pointes) in a structurally normal heart. Prolongation of the QT interval may arise because of a decrease in the K⁺ repolarization currents, an increase in Ca²⁺ entry or to a sustained entry of Na⁺ into the myocyte (ie, increase in I_{Na,t}). An increase in I_{Na,t} is caused by either impaired inactivation, ie, failure to inactivate completely (Figure 3A) or an increase in “window current.” The latter is generally attributable to a shift of voltage dependence of steady-state inactivation toward more depolarized membrane potentials leaving an increased “window” of inward sodium current (Figure 3B).

Until now, 13 different types of LQTS have been described, most of them associated to K⁺ channel disorders. However, a 10% to 15% of the LQTS cases are related to mutations in SCN5A (type 3 LQTS) or in Na current–associated proteins. Patients with type 3 LQTS present arrhythmias associated with bradycardia and symptoms at rest, especially during sleep. Whether β-blockers are as important in type 3 LQT patients as they are in LQT type 1 and 2 patients has been questioned. Data to support nonresponsiveness lack but, preliminary data on a large cohort of LQTS type 3 patients suggest a beneficial effect (A Wilde et al, unpublished data). The best therapy for type 3 LQT patients is currently unknown, but high risk patients are probably those who are symptomatic and those with the longest QT intervals (>500ms). In the latter category a prophylactic ICD might be considered. Gene-specific therapies include sodium channel blockers like flecainide and mexiletine although detrimental attempts, with pathophysiological explanations based on sound experimental studies, have been published. Furthermore, ranolazine, a
new drug with late sodium current blocking efficacy, has been shown to be able to shorten the QTc interval significantly. As many different mutations lead to an overlap syndrome, ie, families with both gain-of-function and loss-of-function phenotype the use of flecainide should be accompanied with serial ECGs because a BrS type ECG (with the proarrhythmogenic substrate) may be the result. For all of these drugs, long-term follow-up is not available.

Dilated Cardiomyopathy
Dilated cardiomyopathy is a cardiac structural disease characterized by decreased systolic function and ventricular dilatation. An average 20% of cases are thought to be familial, most associated with mutations in the cytoskeletal...
proteins. The identification of a mutation in SCN5A in a family with dilated cardiomyopathy provided the first evidence of the disease being caused by an alteration in a cardiac ion channel.

Since then, other publications have confirmed this association. The phenotype is usually associated with arrhythmias (atrial and ventricular arrhythmias and conduction disease).

**Atrial Fibrillation**

AF is defined as an unpredictable activation of the atria, characterized by irregular fibrillatory ECG waves causing an irregular ventricular response, which manifests clinically as an irregular pulse. AF is one of the most common and yet challenging arrhythmias encountered in clinical practice. The prevalence of 1% among the general population increases to 10% among individuals older than 80 years, and it is responsible for more than one-third of all cardioembolic episodes.

Although familial forms had remained mostly unknown, the identification in 1997 of a genetic locus causing familial AF, which defined AF in a subset of patients as a genetic disease with an autosomal dominant pattern of inheritance, initiated further research into the understanding of the pathophysiology of this inherited form of the arrhythmia.

To date, most of the genes associated to AF encode K⁺ channel proteins (KCQ1, KCN2, KCN3, KCNA5, KCN2, and KCNH2). The role of Na⁺ channel proteins in AF has become evident in these last few years. In 2008, several genetic variants in SCN5A, causing both loss and gain of $I_{Na}$, have been associated with lone AF. Most of these phenotypes do not show alterations in the QT interval or in the ST segment elevation. A family with AF with a combined phenotype of LQTS type 3 has been recently described. In addition, mutations in the β-subunits SCN1B, SCN2B, and SCN3B have been associated with AF by decreasing $I_{Na}$. Most of these phenotypes do not show alterations in the QT interval or in the ST segment elevation. A family with AF with a combined phenotype of LQTS type 3 has been recently described. In addition, mutations in the β-subunits SCN1B, SCN2B, and SCN3B have been associated with AF by decreasing $I_{Na}$. It is hypothesized that loss of $I_{Na}$ function leads to altered atrial conduction parameters, increased fibrosis, and associated structural abnormalities (including increased diastolic ventricular pressure in the setting of dilated cardiomyopathy with subsequent enlargement of the atria), that all act together to cause AF.

**Sick Sinus Syndrome**

Diseases of the sinoatrial node (brady-tachy syndrome, sinus bradycardia, sinus arrest) have been associated with loss-of-function mutations in SCN5A, especially severe in patients with compound heterozygote mutations. Similar with other loss-of-function phenotypes, families may show important overlapping features, with family members displaying sick sinus syndrome, whereas others may display BrS or progressive cardiac conduction disease.

Atrial standstill has also been described in SCN5A families. A combined loss-of-function mutation with a loss-of-function connexin 43 polymorphism also leads to atrial standstill.

**Genetic Modulators in Na Current and Sudden Cardiac Death**

Modulation of the phenotype may be related to the presence of environmental factors (ie, inducers like fever in BrS or medications in LQTS) or additional genetic factors. Among these genetic factors, it is well accepted that the presence of compound heterozygotes will confer a worse prognosis in family members. As such, compound heterozygotes involving genes associated with Na current have been described in LQTS, BrS, and sick sinus syndrome.

Polymorphisms have recently acquired more importance in the explanation of certain phenotypes of genetic diseases. Genetic variants in the SCN5A promoter region may have a pathophysiologic role in BrS. A haplotype of 6 polymorphisms in the SCN5A promoter has been identified and functionally linked to a reduced expression of the sodium current. This variant was found among patients of Asian origin, and it could play a role in modulating the expression of BrS in far eastern countries.

In addition, the common H558R polymorphism has been shown to partially restore the sodium current impaired by other simultaneous mutations causing either cardiac conduction disturbances (T512I) or BrS (R282H).

The common variant H558R seems to be a genetic modulator of BrS among carriers of an SCN5A mutation, in whom the presence of the less common allele makes BrS less severe (shorter QRS complex duration in lead II, lower J-point elevation in lead V2, and a trend toward fewer symptoms).

Finally, the presence of the genetic variant S1103Y, which accelerates sodium channel activation, has been associated with arrhythmias and sudden cardiac death in blacks. Of interest in experimental settings, the late sodium current was exacerbated by lowering intracellular pH, making it an important mechanism predisposing a Y1103 carrier to sudden infant death syndrome (SIDS). Indeed, this variant has been identified in black SIDS cases.

**Proteins Associated With Arrhythmias**

Mutations in all 4 β-subunit–encoding genes are found in individuals with various arrhythmic phenotypes, reflecting their importance in normal cardiac electric activity. Interestingly, most such mutations are located in the extracellular amino terminus of the β-subunits (Table 2 and Figure 4), suggesting this domain may play an important role in the interaction and regulation of Na$_{1.5}$ by the β-subunits.

**β1-Subunit**

Two splice variant transcripts of SCN1B, generating β1 and β1 B proteins, are expressed in the heart. The β1-subunit coimmunoprecipitates with Na$_{1.5}$ and is found to colocalize with Na$_{1.5}$ already within the endoplasmic reticulum, suggesting that they mediate the transport of the channel complex to the sarcolemma. The β1-subunit is believed to interact with Na$_{1.5}$ at multiple sites. An interaction between the extracellular domain of the β1-subunit with the extracellular loops between segments 5 and 6 of domains III and IV (SS-S6 loops of DIII and DIV) of Na$_{1.5}$ is required for the regulation of channel gating. In addition, an interaction may also exist between the carboxyl terminus of these 2 subunits.

Accordingly, the p.D1790G mutation in the carboxyl terminus of Na$_{1.5}$, discovered in a large family with LQT-3 has been shown to affect the inactivation of the cardiac Na$^+$.
Mutations in Cardiac Sodium Channel β-Subunits Linked to Arrhythmic Phenotypes

<table>
<thead>
<tr>
<th>β-Subunit</th>
<th>Nucleotide Change</th>
<th>Variant</th>
<th>Mutation Type</th>
<th>Location</th>
<th>Phenotype(s)</th>
<th>Effect of ( i_{Na} )</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>β1</td>
<td>536G&gt;A</td>
<td>W179X</td>
<td>Nonsense</td>
<td>C terminus</td>
<td>Brugada syndrome and cardiac conduction disease</td>
<td>Decrease in peak ( i_{Na} ) and positive shift of activation</td>
<td>74</td>
</tr>
<tr>
<td>β1</td>
<td>537G&gt;A</td>
<td>W179X</td>
<td>Nonsense</td>
<td>C terminus</td>
<td>Cardiac conduction disease</td>
<td>Decrease in peak ( i_{Na} )</td>
<td>74</td>
</tr>
<tr>
<td>β1</td>
<td>259G&gt;C</td>
<td>E87Q</td>
<td>Missense</td>
<td>N terminus</td>
<td>Cardiac conduction disease</td>
<td>Decrease in peak ( i_{Na} ) and positive shift of activation</td>
<td>74</td>
</tr>
<tr>
<td>β1</td>
<td>254G&gt;A</td>
<td>R85H</td>
<td>Missense</td>
<td>N terminus</td>
<td>Atrial fibrillation</td>
<td>Decrease in peak ( i_{Na} ) and positive shift of activation</td>
<td>48</td>
</tr>
<tr>
<td>β1</td>
<td>457G&gt;A</td>
<td>D153N</td>
<td>Missense</td>
<td>N terminus</td>
<td>Atrial fibrillation</td>
<td>Decrease in peak ( i_{Na} )</td>
<td>48</td>
</tr>
<tr>
<td>β2</td>
<td>82C&gt;T</td>
<td>R28W</td>
<td>Missense</td>
<td>N terminus</td>
<td>Atrial fibrillation</td>
<td>Decrease in peak ( i_{Na} ) and positive shift of activation</td>
<td>48</td>
</tr>
<tr>
<td>β2</td>
<td>83G&gt;A</td>
<td>R28Q</td>
<td>Missense</td>
<td>N terminus</td>
<td>Atrial fibrillation</td>
<td>Decrease in peak ( i_{Na} ) and positive shift of activation</td>
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<tr>
<td>β3</td>
<td>17G&gt;A</td>
<td>R6K</td>
<td>Missense</td>
<td>N terminus</td>
<td>Lone atrial fibrillation</td>
<td>Decrease in peak ( i_{Na} )</td>
<td>47</td>
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<tr>
<td>β3</td>
<td>29T&gt;C</td>
<td>L10P</td>
<td>Missense</td>
<td>N terminus</td>
<td>Brugada syndrome</td>
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<td>47, 82</td>
</tr>
<tr>
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<td>161T&gt;G</td>
<td>V54G</td>
<td>Missense</td>
<td>N terminus</td>
<td>Idiopathic ventricular fibrillation</td>
<td>Decrease in peak ( i_{Na} ) and positive shift of activation</td>
<td>79, 84</td>
</tr>
<tr>
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<td>106G&gt;A</td>
<td>V36M</td>
<td>Missense</td>
<td>N terminus</td>
<td>Sudden infant death syndrome</td>
<td>Decrease in peak ( i_{Na} ) and positive shift of activation</td>
<td>84</td>
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<tr>
<td>β3</td>
<td>389C&gt;T</td>
<td>A130V</td>
<td>Missense</td>
<td>N terminus</td>
<td>Atrial fibrillation</td>
<td>Decrease in peak ( i_{Na} ) and increase in persistent ( i_{Na} )</td>
<td>49</td>
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<td>β3</td>
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<td>L161T</td>
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<td>Lone atrial Fibrillation</td>
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<td>β4</td>
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<td>L179F</td>
<td>Missense</td>
<td>Transmembrane segment</td>
<td>Long QT syndrome type 10</td>
<td>Increase in persistent ( i_{Na} ) and positive shift of inhibition</td>
<td>85</td>
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<td>β4</td>
<td>671G&gt;A</td>
<td>S206L</td>
<td>Missense</td>
<td>C terminus</td>
<td>Sudden infant death syndrome</td>
<td>Increase in persistent ( i_{Na} ) and positive shift of inhibition</td>
<td>84</td>
</tr>
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</table>

Figure 4. Schematic representation of the β-subunits of cardiac sodium channel (β1 [A], β2 [B], β3 [C], and β4 [D]), each containing 1 transmembrane segment. For each β-subunit, identified variants that have been linked to either syndrome (see color code below the schematic illustrations) are depicted.

channel by disrupting the interaction between Na\(_{\text{v1.5}}\) and β1-subunit.68 In heterologous expression systems, coexpression of SCN5A with SCN1B have demonstrated variable effects of β1-subunit on gating, depending on the expression system used. However, in most cases, an increase in peak \( i_{Na} \) amplitude,69,70 a shift of steady-state inactivation toward more positive potentials,63,71 and a decrease in persistent \( i_{Na} \) amplitude has been observed.71,72 Surprisingly, ventricular myocytes from SCN1B knockout mice showed increased expression of Na\(_{\text{v1.5}}\) on mRNA and protein level, an increase in peak \( i_{Na} \) amplitude, and no change in Na\(_{\text{v1.5}}\) gating.73 These findings are not in agreement with those observed in in vitro experiments and suggest that heterologous expression systems may not mimic the intracellular environment (in particular the protein–protein interactions) present in cardiac myocytes. However, the SCN1B knockout mice also displayed lower heart rates and repolarization delay (reflected by prolonged QTc and action potential durations), and this may result from an increase in persistent \( i_{Na} \) attributable to the absence of the suppressive effects of β1-subunit on this current. Accordingly, individuals with the previously mentioned D1790G mutation in Na\(_{\text{v1.5}}\), which disrupts the interaction of Na\(_{\text{v1.5}}\) with the β1-subunit, also experienced sinus bradycardia and QTc prolongation.68

Mutations in SCN1B have been found in individuals with BrS, cardiac conduction disease (CCD), or AF. In a recent study by Bezzina and colleagues, 282 probands with BrS and 44 with CCD were screened for mutations in SCN1B.74 A nonsense mutation (c.536G>A resulting in p.W179X) was identified in 4 related individuals, of whom 3 displayed signs of BrS and CCD on their ECGs. This mutation is predicted to
produce a prematurely truncated protein lacking the membrane-spanning segment and the carboxyl terminus. A different nonsense mutation (c.537G>A resulting in p.W179X) was identified in a father and his daughter, of whom only the daughter displayed signs of CCD (right bundle branch block and prolonged PR interval). In both individuals, BrS was unlikely, as established by a negative sodium channel drug challenge test. Finally, a missense mutation (c.259G>C resulting in p.E87Q), located in the amino terminus of the β1-subunit, was found in 3 family members, of whom 2 displayed signs of CCD on their ECGs (complete left bundle branch block, right bundle branch block, and left anterior hemiblock). It must be noted that the families in this study were too small to perform linkage analysis between the phenotypes and the mutations, and that the mutations were also found in individuals with no phenotype. In another study, Roden and colleagues screened 842 AF patients for mutations in SCN1B to SCN4B and identified a mutation in SCN1B in 2 unrelated individuals. The mutations (c.254G>A resulting in p.R85H, and c.457G>A resulting in p.D153N) were both located in the amino terminus of the β1-subunit. Coexpression of all of these mutant β1-subunits with Nav1.5 in CHO cells resulted in a decrease of peak \( I_{\text{Na}} \) density compared with coexpression of Nav1.5 with normal β1-subunits, suggesting that β1-subunits increase the sarclemmal expression of Na\textsubscript{v}1.5 in cardiac myocytes. In addition, p.W179X, p.E87Q, and p.R85H also caused a shift of steady-state activation toward more positive potentials, indicating delayed activation of the channels. These findings are in line with the \( I_{\text{Na}} \) reduction found for virtually all SCN5A mutations related to BrS and CCD, and for few SCN5A mutations related to AF. An \( I_{\text{Na}} \) reduction is speculated to predispose to AF by slowing the conduction velocity of electric stimuli through the atria, thereby facilitating the maintenance of reentrant excitation waves.

**β2-Subunit**

The β2-subunit, encoded by SCN2B, coimmunoprecipitates with Nav1.5. Coexpression of Na\textsubscript{v}1.5 with the β2-subunit resulted in a shift of the steady-state activation toward more positive potentials compared with Nav1.5 expression alone. A similar effect was observed when Na\textsubscript{v}1.5 was coexpressed with the intracellular domain of the β2-subunit, suggesting that this portion of the protein may interact with Na\textsubscript{v}1.5 to modulate the gating. A recent study revealed that the effect of the β2-subunit on Na\textsubscript{v}1.5 activation depends on the presence of sialic acid residues on the β2-subunit (ie, the extent of β2-sialylation). When completely sialylated, β2-subunit caused a reverse shift of the steady-state of activation, ie, toward more negative potentials.

In the above-mentioned study by Roden and colleagues, mutations in SCN2B were identified in 2 unrelated individuals with AF. The mutations (c.82C>T resulting in p.R28W, and c.83G>A resulting in p.R28Q) were located in the amino terminus of the β2-subunit, and their coexpression with Na\textsubscript{v}1.5 resulted in a decrease of peak \( I_{\text{Na}} \) density and a shift of steady-state activation toward more positive potentials compared with when Na\textsubscript{v}1.5 was coexpressed with normal β2-subunits. Both of these effects led to \( I_{\text{Na}} \) reduction, and, accordingly, the individuals carrying the mutations were reported to display electrocardiographic BrS-like changes. Although β2-subunits were previously shown not to colocalize with Na\textsubscript{v}1.5 in the endoplasmic reticulum, indicating they are transported separately to the sarcolemma, the decrease in peak \( I_{\text{Na}} \) density by the mutant β2-subunits suggests that β2-subunits play a role in controlling the sarcolemma expression of Na\textsubscript{v}1.5.

**β3-Subunit**

The β3-subunit, encoded by SCN3B, coimmunoprecipitates with Na\textsubscript{v}1.5. An initial study showed that the β3-subunit is highly expressed in the ventricles and Purkinje fibers but not in atrial tissue of sheep hearts. However, recently, the β3-subunit expression was demonstrated in mouse atrial and ventricular tissues. Compared with Na\textsubscript{v}1.5 alone, coexpression with the β3-subunit resulted in increase in peak \( I_{\text{Na}} \) amplitude and a shift of steady-state inactivation toward more positive potentials (in Xenopus oocytes), or no change in peak \( I_{\text{Na}} \) amplitude and a shift of steady-state inactivation toward more negative potentials (in CHO cells and in HEK-293 cells). These findings illustrate again that the regulatory effects of β-subunits on Na\textsubscript{v}1.5 may vary significantly between heterologous expression systems, probably because of differences in intracellular environments of various expression systems and conditions in which the cells are cultured (eg, lower culture temperature for Xenopus oocytes). Interestingly, coexpression of Na\textsubscript{v}1.5 with β1- and β3-subunits caused an additional shift of inactivation toward more negative potentials, suggesting that β-subunits may act synergistically to regulate gating. Compared with wild-type mice, SCN3B knockout mice displayed slower heart rates, longer P wave durations, and prolonged PR intervals on their ECGs, indicating slowing of electric conduction in the heart. In addition, electrophysiological studies demonstrated inducibility of atrial tachycardia, AF, and ventricular tachycardia in hearts of SCN3B knockout mice, indicating increased atrial and ventricular arrhythmogenecness. Ventricular myocytes isolated from SCN3B knockout mice hearts showed a decrease in peak \( I_{\text{Na}} \) density and a shift of steady-state inactivation toward more negative potentials compared with ventricular myocytes isolated from wild-type hearts. These data are in agreement with those obtained from coexpression studies of β3-subunit with Na\textsubscript{v}1.5 in Xenopus oocytes.

To date, 4 mutations in SCN3B have been linked to arrhythmic phenotypes in humans. Using a candidate gene approach, Antzelevitch and colleagues identified a missense mutation in SCN3B (c.29T>C resulting in p.L10P, located in the amino terminus of β3-subunit) in a patient displaying the ECG signs of BrS. By a similar approach, Makielski and colleagues identified a missense mutation (c.161T>C resulting in p.V54G, also located in the amino terminus of β3-subunit) in a patient with aborted cardiac arrest, attributable to documented ventricular fibrillation, in the absence of structural heart disease or any well-defined arrhythmic phenotype. Coexpression studies revealed that both these mutations cause a decrease in peak \( I_{\text{Na}} \) density and a shift in steady-state inactivation compared with coexpression of
Na,1.5 with normal β3-subunits. Additionally, immunocytochemistry revealed that the mutant β3-subunits decreased the expression of Na,1.5 by disrupting its transport from the endoplasmic reticulum to the sarcolemma. It should be noted that in both cases, there is not much evidence for loss-of-function of cardiac sodium channel activity as even in the presence of procainamide conduction intervals are within normal limits, whereas with SCN5A loss-of-function mutations, conduction is generally prolonged at different levels.

Recently, the p.V54G mutation was also linked to SIDS. Makielski and colleagues screened 292 SIDS cases for mutations in β-subunit–encoding genes and found a mutation in SCN3B in 2 cases (V54G, and c.106G>A resulting in p.V36M, located in the amino terminus). Coexpression of Nav1.5 with p.V36M β3-subunit resulted in a decrease in peak $I_{Na}$ density but, interestingly, also in an increase of the persistent $I_{Na}$ compared with coexpression with normal β3-subunits, showing that mutations in β3-subunits may also cause $I_{Na}$ gain of function. Taken together, findings from these translational studies are largely in line with those obtained from in vitro experiments and SCN3B knockout mice and indicate that β3-subunit plays an important regulatory role in the sarcolemmal expression and gating of Na,1.5. However, recently, Wang and colleagues sequenced 477 patients with AF for mutations in SCN3B and identified a missense mutation in SCN3B (c.389C>T resulting in p.A130V, located in the distal portion of the amino terminus) in one patient. When coexpressed with Na,1.5, the mutant β3-subunit resulted in a decrease in peak $I_{Na}$ density and no gating changes. Surprisingly, Western blot analysis revealed that the mutation did not affect the surface expression of Na,1.5. Based on these data, the authors speculated that p.A30V may decrease $I_{Na}$ by impairing the conductance, but not the intracellular trafficking, of Na,1.5. Although this is an interesting hypothesis, it requires further experimental investigation. Recently, 3 additional loss-of-function mutations in SCN3B have been associated with lone AF (R6K, L10P, M161T). It must be noted that all SCN3B mutations linked to arrhythmic phenotypes are located in the amino terminus of the β3-subunit, suggesting this portion of the protein is important for its regulatory role in the expression and gating of Na,1.5.

### β4-Subunit

The β4-subunit is encoded by SCN4B and communoprecipitates with Na,1.5, and its expression in the heart has been demonstrated on both mRNA and protein levels. Coexpression of Na,1.5 with the β4-subunit did not change peak $I_{Na}$ amplitude but shifted the steady-state inactivation toward more negative potentials compared with expression of Na,1.5 alone. Recently, the β4-subunit has been proposed as a potential genetic modifier of conduction slowing in individuals with cardiac Na$^+$ channel loss of function. This study aimed to assess the role of genetic modifiers on the variable severity of conduction slowing in patients with the SCN5A p.1795insD mutation. To investigate the effect of genetic background without the confounding influences of environmental factors, 2 different mouse strains (FVB/N and 129P2), both carrying the mouse homolog of the p.1795insD mutation, were studied. Similar to the human phenotype, both transgenic mice strains displayed bradycardia and conduction slowing. However, 129P2 mice displayed more severe conduction slowing. Pan-genomic mRNA expression profiling and subsequent Western blot analysis revealed a decrease in SCN4B mRNA and β4-subunit protein levels, respectively, in 129P2 mice compared with FVB/N mice. In addition, measurements of $I_{Na}$ in ventricular myocytes did not find differences in peak $I_{Na}$ but uncovered a shift of steady-state activation toward more positive potentials in 129P2 mice compared with FVB/N, indicating delayed opening of the Na$^+$ channels. Finally, computer simulation models predicted that this delay in activation may be responsible for the differences in conduction slowing between the mouse strains. This report first recognizes a β-subunit of the cardiac Na$^+$ channel as a genetic modifier of phenotype severity in individuals with a mutation in SCN5A.

The importance of the β4-subunit is further reflected by recent studies linking mutations in SCN4B to LQTS (LQT10) and SIDS. Ackerman and colleagues identified a mutation (c.535C>T resulting in p.L179F, located in the transmembrane segment of the β4-subunit) in a LQTS family and a history of unexpected and unexplained sudden deaths. Coexpression of Na,1.5 with mutant β4-subunits resulted in an increase of persistent $I_{Na}$ and a shift of steady-state inactivation toward more positive potentials compared with coexpression of Na,1.5 with normal β4-subunits. In the above-mentioned study on 272 SIDS cases, a mutation in SCN4B (c.671G>A resulting in p.S206L, located in the carboxyl terminus) was also identified. Coexpression experiments in HEK-293 cells showed that, similar to p.L179F, the p.S206L mutation also resulted in an increase in persistent $I_{Na}$ and a shift of steady-state inactivation toward more positive potentials. Accordingly, adult rat ventricular myocytes, adenosinically transduced with the p.S206L mutant β4-subunits, displayed an increase in persistent $I_{Na}$ and prolongation of action potential duration compared with myocytes transduced with the wild-type β4-subunits. Taken together, effects of these 2 SCN4B mutations on the persistent $I_{Na}$ and Na,1.5 gating are in line with effects previously described for SCN5A mutations related to LQTS.

### Glycerol 3 Phosphate Dehydrogenase 1-Like Protein

Approximately 20% of BrS cases have been linked to mutation in SCN5A (see below), and only in a few cases have mutations in β-subunit–encoding genes been found. In search for other genetic causes, London and colleagues linked a multigenerational BrS family to a locus at chromosome 3p22-24 and excluded a disease-causing mutation in SCN5A by linkage and direct sequencing. In a further study, the authors identified a missense mutation (c.899C>T resulting in p.A280V) in GPD1-L, located within the previously found locus, encoding the glycerol-3-phosphate dehydrogenase 1-like protein (GPD1-L). This protein displays 84% homology with the glycerol-3-phosphate dehydrogenase 1 (GPD1), a cytoplasmic enzyme that catalyzes the dehydrogenation of glycerol-3-phosphate (G3P) to dihydroxyacetone phosphate (DHAP) in the glycolytic pathway and the reduction of
DHAP to G3P in the synthesis of triglycerides. GPD1-L was shown to be expressed abundantly in the heart. Compared with coexpression of Na\(_{\text{L}}\) with wild-type GPD1-L, its coexpression with p.A280V GPD1-L resulted in a marked decrease of peak \(I_{\text{Na}}\) density and reduction of cell surface expression of Na\(_{\text{L}}\). In another study, Ackerman and colleagues screened a large cohort of SIDS cases for mutations in GPD1-L. They found 3 novel missense mutations in GPD1-L (c.307G>A resulting in p.E83K, c.430A>G resulting in p.N124V, and c.877C>T resulting in R273C). Similar to the BrS-linked p.A280V mutation, coexpression of Na\(_{\text{L}}\) with each of these mutations cells caused a decrease in peak \(I_{\text{Na}}\) density. Accordingly, adenovirus-mediated transduction of neonatal mouse myocytes with p.E83K GPD1-L reduced \(I_{\text{Na}}\) compared with when myocytes were transduced with wild-type GPD1-L. Based on these data, GPD1-L was suggested to play a role in the intracellular trafficking of Na\(_{\text{L}}\) to the sarcolemma.

More recently, Makielski and colleagues demonstrated in HEK-293 cells that, like GPD1, GPD1-L catalyzes the reaction of G3P to DHAP, and that the BrS-linked p.A280V mutation and the SIDS-linked p.E83K mutation reduce the enzymatic activity of GPD1-L. Next, they showed that reduced GPD1-L activity results in an increased phosphorylation of Na\(_{\text{L}}\) at residue S1503, and this phosphorylation is previously recognized to decrease \(I_{\text{Na}}\). The authors hypothesized that GPD1L loss of function increases the substrate G3P and feeds the protein kinase (PK)C-mediated phosphorylation of Na\(_{\text{L}}\). Indeed, additional experiments, whereby PKC activity was stimulated by G3P or inhibited by staurosporine, indicated that Na\(_{\text{L}}\) phosphorylation and subsequent \(I_{\text{Na}}\) decrease occur through a G3P-dependent PKC-mediated pathway. Although this is an interesting model, future studies confirming these findings in a more native environment are required. Finally, it is worth mentioning that, by using a GST pull-down assay, Makielski and colleagues also showed a previously unrecognized interaction between Na\(_{\text{L}}\) and GPD1-L, which was not disrupted by mutations in GPD1-L. However, they did not determine the location of this interaction or whether it was direct or mediated by channel interacting proteins.

**Caveolin-3**

Caveolae are small narrow-necked invaginations of the plasma membrane of various types that are enriched with lipids (cholesterol and sphingolipids) and proteins (ion channels, receptors, transporters, and signaling molecules). Caveolins are responsible for the formation of caveolae and the maintenance of their structure. Caveolin-3 is the main isoform expressed in the heart. Coimmunoprecipitation of Na\(_{\text{L}}\) with caveolin-3 has been shown in both rat cardiac myocytes and HEK-293 cells. Immunocytchemistry showed that Na\(_{\text{L}}\) colocalizes with caveolin-3, particularly in the caveolae at the sarcolemma but not intracellularly. Interestingly, Shibata and colleagues have shown that \(\beta\)-adrenergic stimulation of rat ventricular myocytes with isoproterenol, in the presence of a PKA inhibitor, causes an increase in peak \(I_{\text{Na}}\). Importantly, this effect could be mimicked by the application of the stimulatory G-protein α-subunit, Go\(_{\alpha}\), and abolished by the addition of antibodies against Go\(_{\alpha}\) or caveolin-3. These findings suggest that Go\(_{\alpha}\) and caveolin-3 act in conjunction to increase peak \(I_{\text{Na}}\) through a cAMP-independent pathway. The authors speculated that a reservoir of Na\(_{\text{L}}\) may be located in caveolar invaginations, where the proteins are not exposed to the extracellular side of the myocyte. By \(\beta\)-adrenergic stimulation and through a Go\(_{\alpha}\) and caveolin-3–dependent pathway, the caveolar neck will open and allow Na\(_{\text{L}}\) to become functional. In this line of research, it has also been demonstrated that a histidine residue at position 41 of Go\(_{\alpha}\) is critical in the regulation of cAMP-independent increase in \(I_{\text{Na}}\). The exact molecular mechanisms by which Go\(_{\alpha}\) or caveolin-3 directly or indirectly (eg, through adaptor proteins such as dystrophin and ankyrin that are also present in the caveolae) interact with Na\(_{\text{L}}\) and cause an increase in functional \(I_{\text{Na}}\) remain to be unraveled.

Based on the preceding evidence, Vatta et al hypothesized that mutations in caveolin-3 may underlie LQTS. They screened 905 unrelated patients with LQTS for mutations in CAV3, the gene encoding caveolin-3, and identified 4 novel missense mutations (c.233C>T resulting in p.T78M in 3 patients, c.253G>A resulting in p.A85T in 1 patient, c.290T>G resulting in p.F97C in 1 patient, and c.423C>G resulting in p.S141R in 1 patient, LQT9). Three mutations are located in the intramembrane domain, and the p.S141R mutation is located in the cytoplasmic carboxyl terminus of caveolin-3. Compared with the coexpression of Na\(_{\text{L}}\) with wild-type caveolin-3, its coexpression with mutant p.F97C or p.S141R caveolin-3 resulted in an increase in persistent \(I_{\text{Na}}\) with no changes in peak \(I_{\text{Na}}\) density and gating. This is in agreement with the effect of SCN5A mutations linked to LQTS type 3 on Na\(_{\text{L}}\). Importantly, Vatta et al also displayed that both p.F97C and p.S141R caveolin-3 coimmunoprecipitate with Na\(_{\text{L}}\), indicating that the increase in persistent \(I_{\text{Na}}\) is not attributable to loss of interaction between mutant caveolin-3 and Na\(_{\text{L}}\). More recently, Ackerman and colleagues screened 134 SIDS cases for mutation in CAV3 and identified 3 distinct mutations in 3 cases (p.T78M previously linked to LQT9, c.40G>C resulting in p.V14L, located in the amino terminus, and c.236T>G resulting in p.L79R, located in the intramembrane domain). Similar to the LQTS-linked mutations in caveolin-3, coexpression of Na\(_{\text{L}}\) with each of these 3 mutations resulted in a marked increase in persistent \(I_{\text{Na}}\) compared with coexpression with wild-type caveolin-3.

Finally, it is noteworthy that the LQTS patient with the p.F97C mutation in caveolin-3 showed QT prolongation only but reproducibly during \(\beta\)-agonist inhaler therapy for asthma. This phenomenon is in line with the previous experimental data demonstrating that caveolin-3 plays a role in the functional increase in \(I_{\text{Na}}\) on \(\beta\)-adrenergic stimulation and suggests that the mutation-induced persistent \(I_{\text{Na}}\) may also increase during such conditions. **α1-Syntrophin**

The SNTA1-encoded α1-syntrophin (SNTA1) is a member of the dystrophin-associated multiprotein complex that interacts directly with the PDZ domain–binding motif formed by the...
last 3 residues (serine-isoleucine-valine) of the Na\(_{1.5}\) carboxyl terminus.\(^{102}\) SNTA1 contains 4 domains, including 2 pleckstrin homology domains (PH1 and PH2), a PDZ domain within the PH1 domain that interacts with Na\(_{1.5}\), and a syntrophin unique (SU) domain. Previously, SNTA1 had been shown to interact also with the neuronal nitric oxide synthase (nNOS), which is constitutively expressed the heart, and the cardiac isoform of plasma membrane Ca\(^{2+}\)/calmodulin-dependent ATPase (PMCA4b).\(^{103,104}\) Of note, in the presence of SNTA1, PMCA4b acts as a potent inhibitor of NO production by nNOS. Importantly, NO has been demonstrated to increase the amplitude of persistent \(I_{\text{Na}}\) in ventricular myocytes.\(^{105}\) Based on these findings, Makielski and colleagues hypothesized that mutations in SNTA1 may disrupt its interaction with PMCA4b, thereby nullifying the ability of PMCA4b to inhibit nNOS, resulting in increased NO synthesis and increased persistent \(I_{\text{Na}}\).\(^{106}\) They screened 50 unrelated individuals with LQTS for mutations in SNTA1 and identified one missense mutation (p.A390V) in one single individual (LQT12). GST-pull-down assay revealed an interaction between Na\(_{1.5}\) carboxyl terminus, nNOS, PMCA4b, and wild-type SNTA1. The p.A390V mutation selectively disrupted the interaction of PMCA4b with Na\(_{1.5}\) and nNOS, and this was associated with increased nitrosylation of Na\(_{1.5}\) as detected by S-nitrosylation biotin switch assay. Moreover, coexpression of Na\(_{1.5}\), nNOS, PMCA4b, and p.A390V-SNTA1 in HEK293 cells increased the peak and persistent \(I_{\text{Na}}\) and shifted the steady-state inactivation toward more positive potentials resulting in larger inward window current compared with coexpression with wild-type SNTA1. These gain-of-function effects were partially abolished when cells were cultured in the presence of \(N^{\text{6+}}\)-monomethyl-L-arginine (L-NMMA), an NOS inhibitor. Moreover, adenoviral transduction of neonatal mouse myocytes with p.A390V-SNTA1 also increased the persistent \(I_{\text{Na}}\) compared with wild-type SNTA1.\(^{106}\) Taken together, these data indicate that, by acting as a scaffolding protein for Na\(_{1.5}\), nNOS, and PMCA4b, SNTA1 plays a key role in regulating the level of Nav1.5 nitrosylation, and thereby the amplitude of peak and persistent \(I_{\text{Na}}\).

According to this study, Vatta and colleagues identified a missense mutation (p.A257G) in 3 of 39 individuals with LQTS.\(^{107}\) Compared with wild-type SNTA1, p.A257G-SNTA1 increased the peak \(I_{\text{Na}}\) and a shift of steady-state activation toward more negative potentials resulting in larger inward window current in HEK-293 cells and neonatal rat myocytes. Although the p.A257G mutation did not alter the persistent \(I_{\text{Na}}\) its gain-of-function effects are in line with those observed for LQTS type 3-related mutations in SCN5A.\(^{88}\) Finally, mutations in SNTA1 have also been linked to SIDS.\(^{108}\) Ackerman and colleagues screened 292 SIDS cases, and identified 6 distinct SNTA1 mutations (p.G54R, p.P56S, p.T262P, p.S287R, p.T372M, and p.G460S) in 8 cases. Interestingly, when coexpressed with Na\(_{1.5}\) in HEK-293 cells, the p.G54R and p.P56S mutations did not affect \(I_{\text{Na}}\) compared with wild-type SNTA1. The p.S287R mutation increased only the peak \(I_{\text{Na}}\), whereas p.S287R, p.T372M, and p.G460S caused similar gain-of-function effects as the LQTS-linked p.A390V mutation (ie, increase in peak and persistent \(I_{\text{Na}}\) and a shift of steady-state inactivation toward more positive potentials). Because the mutations causing an increase in both peak and persistent \(I_{\text{Na}}\) (p.A390V, p.S287R, p.T372M, and p.G460S) were all located in or near to the PH2 and SU domains of SNTA1, the authors speculated that these mutations disrupt the interaction of SNTA1 with PMCA4b, thereby abolishing its inhibitory effect on nNOS. In contrast, mutations located in the PH1 domain of SNTA1 (the LQTS-linked p.A257G and SIDS-linked p.G54R, p.P56S, p.T262P) may not possess the ability to disrupt this interaction.

### Proteins Not Associated With Arrhythmias But With Specific Interactions With SCN5A

A number of other enzymes and regulatory proteins have been shown to interact with the Na\(_{1.5}\), but, as yet, no disease-related variants in any of these proteins have been identified (Table III and references in the Online Data Supplement, available at http://circres.ahajournals.org). However, for a number of individual mutations in SCN5A, it has been shown that the interaction between Na\(_{1.5}\) and the relevant protein is modified, giving rise to a distinct phenotype. Notable examples are the A1924T mutation in the IQ motif of Nav1.5 that abolishes the enhanced entry of the channels into slow inactivation induced by calmodulin, resulting in reduced cardiac excitability;\(^{109}\) the BrS-linked E1053K in Na\(_{1.5}\) mutation that abolishes ankyrin G binding to Na\(_{1.5}\) and prevents normal channel trafficking to the intercalated discs;\(^{110,111}\) and the above-mentioned LQTS type 3-linked D1790G that abolishes binding of fibroblast growth factor homologous factor 1B, thereby abolishing the negative shift of inactivation.\(^{112}\)

### Conclusion

A short 2 decades of research have raised enormous insight into the role of the cardiac sodium channels and their regulatory subunits in arrhythmogenesis and have led to the description of new disease entities. For some patients with monogenic diseases, tailored therapy has ensued; for others, nothing has changed or therapeutic choices have become even more complicated. Basic insight into the role of all of these proteins assembled in the multiprotein cardiac sodium channel complex has been greatly advanced. Yet, many details are still lacking, among which, for example, which \(\beta\) subunits associate with which \(\alpha\) subunits. Is this only Nav1.5? Furthermore, the spatial, temporal, and developmental distribution of these interaction are only partly known. In addition, it is still incompletely understood why, eg, loss-of-function mutations in SCN5A lead to so many different phenotypes. Gender, age, differential expression throughout the heart, coexpression, eventual tissue specific, and expression of other genetic variants (eg, the connexin variant discussed above) all may play a role, and the interaction of SCN5A is complex but is being increasingly unraveled. In the end, this may lead to a better prospect for all patients with these rare diseases, as well as for those with far more common disease entities, among which are heart failure— or
ischemia-related atrial and ventricular arrhythmias, all with a multifactorial background.

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Phenotypical Manifestations of Mutations in the Genes Encoding Subunits of the Cardiac Sodium Channel
Arthur A.M. Wilde and Ramon Brugada

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Online Table I: Proteins or protein families that have been reported to interact with and regulate Na\textsubscript{v}1.5, but are not (yet) associated with arrhythmias.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Type</th>
<th>Binding region on Nav1.5</th>
<th>Effect on Nav1.5</th>
<th>Effect on I\textsubscript{Na}</th>
<th>Ref</th>
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<td>Nedd4-like enzymes (ubiquitin-protein ligases)</td>
<td>Enzyme*</td>
<td>PY motif in C-terminus</td>
<td>Ubiquitylation and internalization</td>
<td>Decrease in peak I\textsubscript{Na}</td>
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<tr>
<td>14-3-3η</td>
<td>Regulator y protein* #</td>
<td>Cytoplasmic linker DI-II</td>
<td>Negative shift of inactivation</td>
<td>Decrease in I\textsubscript{Na}</td>
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<tr>
<td>Fibroblast growth factor homologous factor 1B (FHF1B)</td>
<td>Regulator y protein</td>
<td>Amino acids 1773-1832 in C-terminus</td>
<td>Negative shift of inactivation</td>
<td>Decrease in I\textsubscript{Na}</td>
<td>5</td>
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<tr>
<td>Calmodulin</td>
<td>Regulator y protein</td>
<td>IQ motif in C-terminus</td>
<td>Positive shift of inactivation (only in Ca\textsuperscript{2+}-free conditions)</td>
<td>Increase in I\textsubscript{Na}</td>
<td>6-7</td>
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<tr>
<td>Ca\textsuperscript{2+}/calmodulin-dependent protein kinase II\textsubscript{bc} (CAMKII\textsubscript{bc})</td>
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<td>Not determined</td>
<td>Phosphorylation</td>
<td>Decrease in I\textsubscript{Na}</td>
<td>8-9</td>
</tr>
<tr>
<td>Protein tyrosine kinase Fyn</td>
<td>Enzyme*</td>
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<td>Phosphorylation</td>
<td>Increase in I\textsubscript{Na}</td>
<td>10</td>
</tr>
<tr>
<td>Protein tyrosine phosphatase H1 (PTPH1)</td>
<td>Enzyme</td>
<td>PDZ domain binding motif in C-terminus</td>
<td>Dephosphorylation</td>
<td>Decrease in I\textsubscript{Na}</td>
<td>11</td>
</tr>
<tr>
<td>Telethonin</td>
<td>Regulator y protein* #</td>
<td>Not determined</td>
<td>Silencing induces:</td>
<td>Increase in I\textsubscript{Na}</td>
<td>12</td>
</tr>
<tr>
<td>Multicopy suppressor of gsp 1 (MOG1)</td>
<td>Adapter protein* #</td>
<td>Cytoplasmic linker DII-III</td>
<td>Increase cell surface expression</td>
<td>Increase in peak I\textsubscript{Na}</td>
<td>13</td>
</tr>
<tr>
<td>Plakophilin-2</td>
<td>Adapter and regulatory protein* #</td>
<td>Not determined</td>
<td>Silencing induces:</td>
<td>Increase in I\textsubscript{Na}</td>
<td>14</td>
</tr>
<tr>
<td>Ankyrin G</td>
<td>Adapter protein* #</td>
<td>Cytoplasmic linker DII-III</td>
<td>Transport to cell surface</td>
<td>Increase in peak I\textsubscript{Na}</td>
<td>15</td>
</tr>
</tbody>
</table>

* In vivo effect has been demonstrated in cardiac tissue.

# Co-localization with Nav1.5 (at the intercalated disk of myocytes): 14-3-3η
References


