Published in final edited form as:
Card Electrophysiol Clin. 2014 December 1; 6(4): 797–809. doi:10.1016/j.ccep.2014.08.007.

Diseases caused by mutations in $Na_v1.5$ interacting proteins

John W. Kyle and **Jonathan C. Makielski, MD**
Department of Medicine, Division of Cardiovascular Medicine, University of Wisconsin, Madison, Wisconsin, USA 53792

Synopsis

Sodium current in the heart flows principally through the pore protein $Na_V1.5$, which is part of a complex of interacting proteins that serve both to target and localize the complex in the membrane, and to modulate function by such post-translational modifications as phosphorylation and nitrosylation. Multiple mutations in seven different $Na_V1.5$ interacting proteins have been associated with dysfunctional sodium current and inherited cardiac diseases, including long QT syndrome, Brugada syndrome, atrial fibrillation, and cardiomyopathy, as well as sudden infant death syndrome (SIDS). Mutations in as yet unidentified interacting proteins may account for cardiac disease for which a genetic basis has not yet been established. Characterizing the mechanisms by which these mutations cause disease may give insight into etiologies and treatments of more common acquired cardiac disease, such as ischemia and heart failure.

Keywords

Arrhythmia; Macromolecular complex; Sodium channel; Long QT syndrome; Brugada syndrome; Sudden Infant Death Syndrome; Cardiomyopathy; Sodium current; Late Sodium Current; SCN5a

Clinical Importance

In this review we summarize current understanding of cardiac diseases caused by mutations in proteins that interact with the cardiac sodium channel $Na_V1.5$. $Na_V1.5$ is encoded by the gene *SCN5A* and forms the pore through which flows the majority of sodium current (I_{Na}). More than 30 sodium channel interacting proteins (SCIPs) have been identified[1, 2] (Table 1). Excluding the four β-subunits, which are covered in Chapter 13, mutations in seven additional SCIPs have been associated with cardiac diseases (Table 2). These diseases include inherited arrhythmia syndromes such as long QT syndrome (LQTS), Brugada syndrome (BrS) and atrial fibrillation (AF), as well as inherited cardiomyopathy. We also include sudden infant death syndrome (SIDS) as a disease where deaths may be presumed to have a cardiac cause and where ~10% have been associated with channelopathies[3].

Corresponding Author: Jonathan C Makielski, MD, Phone 608 263-9648, jcm@medicine.wisc.edu.

Disclosure Statement: No conflicts to disclose

Mutations in SCIPs are a rare cause of inherited arrhythmia (<1% of LQTS and BrS), but they account for ~5% of SIDS overall[3] or half of the SIDS cases associated with channelopathies. Mutations in *SNTA1* alone represent ~25% of SIDS associated channelopathies (~2.5% of SIDS overall). Despite the overall rarity, the study of these mutations has provided insight into the regulation of I_{Na} and the pathophysiology of arrhythmia in common acquired cardiac diseases, such as ischemia and heart failure. In addition, an important percentage of BrS and SIDS remain without a linked genotype and SCIPs, both known (Table 1) or as yet unidentified, could account for the etiology of an important percentage of these syndromes.

Definition of SCIPs and the Sodium Channel Complex (SSC)

SCIPs are defined as proteins that co-localize with $Na_V1.5$ as part of a Sodium Channel Complex (SCC) and where a physical association has been shown by co-immunoprecipitation, GST-fusion protein pull-down, or other binding assay, such as yeast-two hybrid assay. SCIPs have been variously called and classified as accessory proteins, auxiliary proteins, associated proteins, anchoring proteins, β-subunits, scaffolding proteins, adaptor proteins, and regulatory proteins[2, 4–6]. Each term has intuitive implications for structure or function, but the terms are not usually rigorously defined and classifications may overlap. Co-localization may occur through direct interaction with $Na_V1.5$ or indirectly through other SCIPs. For example both α1-syntrophin and ankyrin-G serve to connect SCIPs to $Na_V1.5$ and are called adapter or scaffolding proteins. In many cases the sites of physical interaction are known, and most SCIPs have also been shown to affect I_{Na} function. On the other hand some proteins affect I_{Na} or "interact functionally" but have not been shown to be physically associated with the SCC and do not meet this formal definition of a SCIP. For example, PKC affects $Na_V1.5$ density[7] but has not been shown to be associated with the SCC. A broader definition of SCIPs might include proteins such as chaperones that associate during trafficking to the membrane or tags for recycling/degradation, such as ubiquitin[8], but for this review we use the narrower definition. Another issue with the definition is that through the cytoskeleton and scaffolding proteins the SCC may be attached more distantly to many other complexes in the cell. As the field evolves, more precise definitions of "interacting" based on proximity, time, location, and function will emerge.

Although more than 30 SCIPs have been identified (Table 1), they are not present in every complex at all times. Indeed two distinct "pools" of SSCs have been identified in cardiac myocytes, one at the lateral membrane associated with SNTA1 and a second at the intercalated disc with SAP97/plakophilin-2 (PKP2)[9, 10]. Also, the dynamic nature of the SCC is not known; are the components "permanent" for the ~35 hour half-life[11] of the channel at the cell surface, or are they transient over some time period?

Mutations, disease, and causality

When a mutation is identified in a patient with a particular disease, perhaps the strongest evidence of causality is the strength of genetic linkage analysis. As an example, GPD1L was discovered through a strong genetic linkage to the BrS phenotype[12], however, most mutations in SCIPs do not have this level of genetic linkage evidence for causality. Absence

of a mutation in "control" populations and high conservation of the residue across species provides additional genetic evidence for causality. Plausible pathogenicity of a candidate disease mutation is often built on the functional importance of the mutation by studies at the molecular and cellular level. The mechanisms by which I_{Na} dysfunction causes clinical syndromes is covered in detail in other articles in this issue, but can be briefly conceptualized as a gain of function where I_{Na}, particularly late I_{Na} corresponding to phase 2 and 3 of the action potential is increased, or as "loss of function" where I_{Na}, particularly peak or early I_{Na} corresponding to phases 0 and 1 of the action potential, is decreased.

Scope of this review

We feature five SCIPs that meet the definition above and also include SLMAP and ZASP as two more putative SCIPs (Table 2 and Fig. 1) that have evidence of causation of a cardiac disease through changes in I_{Na}. SCIPs that are not considered include mutations in the SCIP telethonin associated with irritable bowel syndrome[13], but not associated with cardiac disease, which is the scope of this review. A mutation within $Na_V1.5$ disrupted association with the SCIP ankyrin-G in BrS patients[14], but this is not included because no disease causing mutations in ankyrin-G itself have been yet identified. Mutations (D130G and F142L) in the SCIP calmodulin (CALM1) (Table 1) were discovered in infants with long QT arrhythmia[15]; both showed decreased sensitivity to calcium, but effects on I_{Na} are not yet established.

SNTA1 and LQTS/SIDS

SNTA1 or α1-syntrophin is a 54 kDa cytoplasmic membrane-associated adaptor protein that is a member of the multigene syntrophin family and is coded for by the *SNTA1* gene[16]. Syntrophins contain three protein interacting domains: 1) a PDZ domain (postsynaptic density protein-95/disc large/zona occludens-1), 2) a plextrin homology (PH) domain, one of which is split by the PDZ domain in SNTA1, and 3) a syntrophin unique domain (SU) (Fig. 1A). SNTA1 also contains a second intact PH domain following the split PH domain. SNTA1 serves as an adaptor to link proteins with signaling molecules, such as nNOS or calmodulin, or even other adaptor proteins, such as dystrophin.[16] An adaptor protein itself does not possess intrinsic activity, but localization of bound signaling molecules to the microenvironment provides specificity[17]. The PDZ domain on SNTA1 interacts with a PDZ binding domain on the last 3 amino acids of the C-terminus of $Na_V1.5$ as shown by pull-down of SNTA1 by a GST-fusion protein containing 66 amino acids of the $Na_V1.5$ C-terminus (Fig. 2).[18] When the last 3 amino acids (SIV) of the $Na_V1.5$ C-terminus were deleted syntrophins and dystrophins were no longer pulled down.

A screen for mutations in SNTA1 in a cohort of 50 unrelated LQTS patients negative for 11 established LQTS genes yielded a missense mutation (A390V-SNTA) in an 18 year old male with syncope[19]. Previous work on neuronal nitric oxide synthase (nNOS) in brain established a physical association between SNTA1, nNOS, and the plasma membrane calcium ATPase subtype 4b calcium pump (PMCA4b)[20]. Other studies in heart established that PMCA4b inhibited nNOS[21]. SNTA1 also associated with $Na_V1.5$ in heart[18], and together these studies raised the possibility that SNTA1 was part of an SCC in heart with

SNTA1 connecting nNOS and PMCA4b to $Na_V1.5$ (Fig. 2). Confirmation of $Na_V1.5$/SNTA1/nNOS/PMCA4b complex was demonstrated in transfected HEK cells where GST-$Na_V1.5$ C-terminal fusion constructs pulled-down WT-SNTA1, nNOS and PMCA4b. When A390V-SNTA1 was co-expressed instead of WT-SNTA1, the association with PMCA4b was lost[19]. The A390V mutation is within the second PH2 domain in SNTA1 (Fig. 1A & Fig. 2) and is thought to be the stronger of two association sites between PMCA4b and SNTA1, the weaker association being between a PDZ domain on the C-terminus tail of PMCA4b and the PDZ domain within the split PH1 domain on SNTA1[16]. The loss of association of PMCA4b with A390V-SNTA1 resulted in increased S-nitrosylation of $Na_V1.5$ and increased late I_{Na} and both increases were prevented by the addition of L-NMMA, an arginine analogue and specific inhibitor of nNOS[19]. These results support the idea that late I_{Na} was increased secondary to S-nitrosylation of $Na_V1.5$ caused by A390V-SNTA1 disruption of association of the nNOS inhibitor PMCA4b (Fig. 2).

A screen of 39 LQTS patients negative for mutations in known LQTS susceptibility genes identified a single missense mutation in SNTA1 (A257G-SNTA1) in 3 unrelated patients[22]. A257G is in a highly conserved region of SNTA1 and it was not found in controls. Expression studies with A257G-SNTA1 showed increased peak I_{Na} but no change in late I_{Na}. Kinetic effects with co-expression of A257G-SNTA1 included a negative shift of 7–10 mV in steady-state activation. Computer modelling supported the role of A257G-SNTA1 in triggering arrhythmia[22]. It should be noted that the expression studies did not include nNOS or PMCA4b, which were shown[19] to be required for increased late I_{Na} with A390V-SNTA1, and this could explain why no disproportionate increase in late I_{Na} was observed. Together these studies[19, 22] established SNTA1 as a cause of LQTS and was designated LQT12.

SNTA1 was screened for mutations in 282 SIDS cases and 6 rare missense mutations (G54R, P56S, T262P, S287R, T372M and G460S) were found in 8 cases that were absent in 800 controls[23]. When these were co-expressed with $Na_V1.5$, PMCA4b, and nNOS the results for S287R, T372M and G460S were similar to A390V-SNTA showing increased late I_{Na} that was blocked by nNOS inhibitors, thereby establishing mutations in SNTA1 as a plausible cause of SIDS. Missense mutations in both SNTA1 and $Na_V1.5$ (A261V-SNTA and R800L-$Na_V1.5$) were found in a three generation family with LQTS[24]. Co-expression of both mutations showed increased late I_{Na} that was the sum of each mutation expressed alone, suggesting additive effects to produce the phenotype. In another example of interaction, the P74L-SNTA1 polymorphism mitigated the deleterious effect of the LQT12 A257G-SNTA1 mutation[25].

CAV3 and LQTS/SIDS

Caveolin-3 (CAV3) is a small ~17 kDa integral membrane protein highly expressed in skeletal muscle and heart[26–28]. CAV3 is a member of a family of caveolins which are the major proteins of caveolae, cholesterol-enriched invaginations of the sarcolemma (Fig 3). CAV3 mutations (Fig. 1B) were identified in LQTS (classified as LQT9)[29] and SIDS[30] and showed increased late I_{Na} when co-expressed with $Na_V1.5$. CAV3 has also been shown to associate with and inhibit nNOS[31] (Fig. 3), raising the possibility that LQT9, analogous to LQT12, is caused by a loss of function by CAV3 to inhibit nNOS leading to increased S-

nitrosylation of $Na_V1.5$ and increased late $I_{Na.}$. Expression of the LQT9 mutation F97C-CAV3 in both HEK cells and rat myocytes showed increased late I_{Na} and rat myocytes showed increased action potential duration that were abrogated by the NOS inhibitor L-NMMA[32]. Evidence for increased S-nitrosylation of $Na_V1.5$ was provided by experiments in HEK cells where S-nitrosylation of $Na_V1.5$ determined by biotin switch assay was increased by F97C-CAV3 compared to WT-CAV3 [32]. Interestingly F97C-CAV3 remained associated with the SCC but lost the nNOS inhibition activity as determined by enzymatic assay[32]. This suggested that CAV3 and SNTA1 mutations share a common mechanism to increase late I_{Na} by releasing inhibition of nNOS leading to an increase in S-nitrosylation of $Na_V1.5$ (or other SCC) and an increased late I_{Na}. It is important to note that CAV3 interacts with multiple ion channels and transporters, and ion currents other than I_{Na} may also be affected by these mutations. Indeed these mutations decrease the inward rectifier current by decreasing KIR2.1 channel protein expression at the surface[33], and this likely contributes to the pathogenesis of LQT9.

GPD1L and BrS

GPD1L (Glycerol Phosphate Dehydrogenase 1 Like) is a 38 kDa protein (Fig. 1C) with very high homology to human cytoplasmic GPD1(cGPD1)[34], an enzyme that catalyzes the reversible reaction converting dihydroxyacetone phosphate to glycerol 3-phospate with the subsequent oxidation of NADH to NAD^+. cGDP1 is an NAD-dependent cytosolic enzyme that is an important link between the glycolytic pathway and triglyceride synthesis. GPD1L was unknown as a human protein until the mutation A280V-GPD1L was implicated in BrS by linkage analysis and gene walking in a large family[35]. This BrS mutation[12] and three novel SIDS-associated GPDL1 mutations (E83K, I124V and R273C)[36] decreased I_{Na} by >50% when the mutant GPD1L cDNAs were transfected into HEK cells expressing $Na_V1.5$[12, 36]. The SIDS mutations transfected in mouse myocytes[36] also increased late I_{Na}. In heterologous expression systems GPD1L co-localized with $Na_V1.5$ and was shown to be associated with $Na_V1.5$ by co-immunoprecipitation, and $Na_V1.5$ was pulled down by GST-GPD1L (both WT and GPD1L mutants)[37], establishing GPD1L as a SCIP. GPD1L was localized at the cell surface[12] and the A280V mutant decreased cell surface expression ~30%[12], but the mechanism for the decrease was unknown. How does a defect in an enzyme that is presumably involved in metabolic pathways specifically target $Na_V1.5$? Two different but not mutually exclusive mechanisms have been proposed. One hypothesis[37] has the A280V-GPD1L mutation leading to an increased concentration of glycerol-3-phospate which would increase other substrates in the pathway to diacyl-glycerol (DAG). DAG concentration would increase primarily in the area near the NaV1.5/GPD1L SCC and upregulate PKC, which is known to decrease I_{Na} by direct channel phosphorylation at S1503[7]. In support of this hypothesis E83K-SNTA1 and A280V-SNTA1 decreased both I_{Na} and cell surface expression of $Na_V1.5$ and these decreases were abrogated both by pharmacological blockers of the PKC-related pathway and also by co-expression with the PKC phosphorylation deficient $Na_V1.5$ mutant S1503A[37]. This mechanism could account for specificity by co-localization of GPD1L and intermediates with $Na_V1.5$ substrate and direct phosphorylation of $Na_V1.5$. Another proposed mechanism has the mutant increase NADH, and through PKC effects on mitochondria, to increase ROS, which then decreases I_{Na} by unspecified mechanisms[38, 39]. It is not clear however, how this mechanism would

provide specificity for $Na_V1.5$ as a general increase in ROS would have wide effects. Either or both mechanisms could be operant depending upon the direction of the reversible reaction that GPD1L regulates. GPD1L causing BrS is very rare[40] and is only slightly more common in SIDS[3], but of wider interest is linkage of a SNP upstream of GPD1L that has been associated with sudden cardiac death in patients with coronary artery disease[41]. Whether or not this association occurs through $Na_V1.5$ is unknown, but the localization to $Na_V1.5$, the functional interaction, and the genetic associations suggest an important role for GPD1L in regulating cardiac excitability.

MOG1 and BrS/AF

MOG1 is a 20 kDa protein (Fig. 1D) coded for by the *RANGRF* gene (Ran GTP release factor). The yeast homolog, scMOG1, is primarily located in the nucleus and acts as a Ran GTP release factor regulating nuclear import and export of proteins. Human MOG1 binds to both yeast and human Ran, has a GTP release activity that has been mapped to the first 45 amino acids[42] (Fig 1D), and can partially rescue the growth defect in yeast cells lacking scMOG1, showing that it retains some of the activity of the yeast homologue[43]. MOG1 was identified as a SCIP in human heart by a yeast two-hybrid screen using a human heart cDNA library as prey[44] and the second intracellular loop of Nav1.5 between repeats II-III as bait[44]. Although MOG1 is mostly found in the nucleus it also co-localizes with $Na_V1.5$ at the intercalated disc[44]. $Na_V1.5$ and MOG1 co-immunoprecipitation and GST pull-down assays confirmed the association between the $Na_V1.5$ cytoplasmic loop II and in vitro translated MOG1. The GST pull-down assay using in vitro translated proteins is notable because it demonstrates a direct interaction without the need for intermediates to link MOG1 to $Na_V1.5$, although the precise interaction sites are not known. Co-expression of $Na_V1.5$ and MOG1 in HEK cells increased I_{Na} 2-fold without affecting steady-state activation, steady-state inactivation or recovery from inactivation. Similarly, overexpression of MOG1 in mouse neonatal cardiomyocytes increased I_{Na} with no change in single channel conductance, suggesting that the increased I_{Na} was caused by an increase in channel number at the cell surface. Knock-down of MOG1 by siRNA in neonatal mouse cardiomyocytes decreased I_{Na}[45, 45].

A screen of 246 BrS patients[46] yielded a missense mutation (E83D-MOG1) in a female patient with BrS that was absent in controls. Co-expression of E83D-MOG1 with $Na_V1.5$ in HEK cells caused ~50% reduced I_{Na} without a change in kinetics, and it exerted a dominant-negative effect on WT-MOG1 in co-expression experiments. The E61X nonsense mutation causing a premature stop (E61X) was reported in 4 patients in a screen of 197 patients with lone AF and in one of 23 patients with BrS[47], but this mutation was also detected in two control subjects. Expression of E61X-MOG1 with $Na_V1.5$ in CHO-K1 cells showed ~50% decreased I_{Na} but when E61X-MOG1 was co-expressed with WT-MOG1 there was no dominant-negative effect and the levels of I_{Na} were comparable to control. The pathogenicity of E61X was further questioned when it was detected in an asymptomatic patient with a BrS ECG and in five other asymptomatic family members[48].

PKP2 and BrS and Cardiomyopathy

Plakophilin (PKP2) is a 98 kDa protein and a member of a family of desmosomal proteins localized primarily at the intercalated disc in cardiomyocytes. PKP2 has a 335 amino acid N-terminus that is the site of interaction with binding partners (Fig. 1E)[49, 50]. There are 8–9 armadillo repeats, a signature feature of the family, and a characteristic bend occurs between armadillo repeats 5 and 6 followed by a short C-terminus[49, 50]. PKP2 is a scaffolding protein that with other desmosomal proteins forms a bridge between cadherens and intermediate filaments. Mutations in PKP2 have been linked to familial arrhythmogenic cardiomyopathy (AC), also called arrhythmogenic right ventricular cardiomyopathy[51]. As with heart failure[52] and other cardiac diseases, $Na_V1.5$ is often reduced in AC[53]. In autopsy samples from 5 AC patients and 5 normal controls, immunohistochemistry at the intercalated discs showed that levels of $Na_V1.5$, Cx43 and plakoglobin were reduced in most patients (65–74%) while PKP2 was not affected unless there was a PKP2 mutation[53]. These results show that levels of expression of $Na_V1.5$ at the intercalated disc can be reduced independently of PKP2. Evidence that PKP2 is a SCIP was first provided in siRNA knock down experiments of PKP2 in rat cardiomyocytes[54] where PKP2 protein was reduced and I_{Na} reduced ~50% with no apparent loss of total $Na_V1.5$ protein. This suggested a redistribution of NaV1.5 from the cell surface to intracellular locations. Evidence for association between PKP2 and $Na_V1.5$ was provided by pull-down of $Na_V1.5$ by a GST-fusion construct containing the N-terminus of PKP2[54]. The kinetics of $Na_V1.5$ were altered after siRNA knock down of PKP2 with a negative shift in steady-state inactivation and slower recovery from inactivation[54]. Optical mapping showed a slowing of conduction[55] suggesting PKP2 as a potential causative agent in BrS. Subsequently a screen of 200 BrS patients without evidence of AC and negative for mutations in other genes linked to BrS yielded 5 PKP2 missense mutations (Q62K, S183N, M365V, T526A and R635Q)[56] (Fig. 1E). I_{Na} and the number of $Na_V1.5$ channels found at the intercalated disc were decreased when PKP2 mutants were expressed in a rat atrial cell line (HL1 cells), human embryonic stem cell derived cardiomyocytes, or in induced pluripotent stem cell derived cardiomyocytes from an AC patient. A dominant-negative effect was absent when WT and mutant PKP2 were co-expressed. In contrast to PKP2 siRNA knock down studies in cardiomyocytes, sodium channel kinetics were not affected[56]. Single channel properties were also unaffected and taken together with immunohistochemical data are consistent with a reduction of $Na_V1.5$ protein at the intercalated discs[56]. These data support a role for PKP2 in targeting and transport of $Na_V1.5$ to the intercalated disc[56] as recently reviewed[57].

SLMAP and BrS

Sarcolemma membrane-associated protein (SLMAP or SLAP) is a 95 kDa protein that is localized to the sarcolemma and t-tubules near the sarcoplasmic reticulum. SLAMP3, the longest of three alternatively splice forms (37, 46, 74 kDa respectively) is predominant in heart (Fig. 1F)[58]. SLMAP and ZASP (see below) are recently identified disease causing SCIPs included in this review despite falling outside the strict definition of a disease causing SCIP put forth earlier. A screen of 190 unrelated BrS patients identified two missense mutations (V269I and E710A) in SLMAP[59]. Both mutations decreased I_{Na} and also cell surface expression of both SLMAP and $Na_V1.5$ in HEK cells, with no change in I_{Na} gating kinetics. SLMAP and $Na_V1.5$, failed to co-immunoprecipitate suggesting that SLMAP may

not be a tightly associated part of the SCC [59]. SLMAP may play a role in targeting of $Na_V1.5$ and is a candidate for further study as a BrS linked gene, but it has not yet been firmly established as a SCIP.

ZASP and Dilated Cardiomyopathy/Left Ventricular Noncompaction Syndrome

ZASP (Z-band alternatively spliced PDZ motif protein) is encoded by the gene *LDB3Z4* (Lim Domain-Binding Protein 3) and is a member of a group of 10 genes that code for proteins that contain both PDZ and multiple LIM domains (Fig. 1G)[60]. Multiple alternative spliced forms of ZASP exist and the protein coded by longest transcript is 77 kDa (Table 2). All alternatively spliced forms of ZASP have an N-terminal PDZ domain but some forms lack the C-terminal Lim domains (Table 2). LIM domains contain a cysteine-rich consensus Zinc-finger sequence and are protein interaction domains[60]. Mutations in ZASP have been identified in patients with dilated cardiomyopathy and left ventricular non-compaction[61] and cardiac specific loss of the murine ZASP homologue results in a severe dilated cardiomyopathy and premature death[62]. In addition to a role in stabilizing the sarcomere structure, ZASP can act as an adaptor protein as, for example, to bridge alpha-actinin-2 through its N-terminal PDZ domain with PKC or PKA through its C-terminal LIM domains[5] or to bridge the L-type calcium channel through its C-terminal PDZ binding motif with PKA through the LIM binding domain[5]. Mutations in ZASP have been associated with myofibrillar myopathy, and dilated cardiomyopathy[61]. D117N-ZASP, a mutation reported previously[61], was found in a patient with Left Ventricular Noncompaction Syndrome[63] and when co-expressed with $Na_V1.5$ in HEK293 cells and in rat neonatal myocytes it caused a ~30% reduction in I_{Na}. Steady-state activation was shifted +9 mV in HEK cells and +15 mV in myoctes and inactivation was shifted by + 4 mV in HEK cells and +10 mV in myocytes, and recovery from inactivation was slowed[63]. Computer modeling supported the hypothesis that the reduction in I_{Na} mediated by ZASP-D117N would generate arrhythmias. Association with Nav1.5 was demonstrated by co-immunoprecipitation using purified ZASP (or D117N- ZASP) produced in E. coli. mixed with homogenates from $Na_V1.5$ transfected HEK cells or myocytes[63]. The reported association of ZASP with $Na_V1.5$ [63] and subsequently with L-type calcium channels [5] acts as a reminder that SCIPs can be promiscuous (see also Cav3) and the pathogenesis may be complex.

Summary

SCIPs serve multiple functions including targeting of the SCC to the sarcolemma and regulating function through such mechanisms as post-translational modification (phosphorylation and nitrosylation). Specificity to $Na_V1.5$ and I_{Na} can be achieved by directly interacting with the $Na_V1.5$ channel protein but also by localizing signaling pathway components to the local milieu [17]. When this regulation is disturbed by mutations in SCIPs the resulting dysregulation of I_{Na} can be a mechanism for diseases such as inherited arrhythmia syndromes and SIDS. Although at present mutations in SCIPs are a relatively rare cause of cardiac disease, they are prime candidates to account for BrS syndrome and other inherited arrhythmia syndromes, as well as SIDS and cardiomyopathies, where genetic causes are suspected but not yet demonstrated. At a more basic level, understanding the mechanisms of how mutations in SCIPs cause disease may give insight into the etiology and

treatment options of the more common acquired cardiac diseases, including the contribution of subtle genetic variations as susceptibility variants to cardiac disease.

Acknowledgments

The authors thank Maeve Makielski for Figure artwork.

Reference List

1. Adsit GS, Vaidyanathan R, Galler CM, Kyle JW, Makielski JC. Channelopathies from mutations in the cardiac sodium channel protein complex. J Mol Cell Cardiol. 2013
2. Abriel H. Cardiac sodium channel Na(v)1. 5 and interacting proteins: Physiology and pathophysiology. J Mol Cell Cardiol. 2010; 48(1):2–11. [PubMed: 19744495]
3. Makielski, JC. SuddenInfant Death Syndrome. In: Zipes, DP.; Jalife, J., editors. Cardiac Electrophysiology: From Cell to Bedside. 6. Philadelphia: Elsevier; 2014. p. 975-980.
4. Isom LL, De Jongh KH, Catterall WA. Auxiliary subunits of voltage-gated ion channels. Neuron. 1994; 12:1183–1194. [PubMed: 7516685]
5. Lin C, Guo X, Lange S, et al. Cypher/ZASP is a novel A-kinase anchoring protein. J Biol Chem. 2013; 288(41):29403–29413. [PubMed: 23996002]
6. Vaidyanathan, R.; Makielski, JC. Scaffolding proteins and ion channel diseases. In: Zipes, DP.; Jalife, J., editors. Cardiac Electrophysiology: From Cell to Bedside. 6. Philadelphia: Elsevier; 2014. p. 229-234.
7. Murray KT, Hu NN, Daw JR, et al. Functional effects of protein kinase C activation on the human cardiac Na+ channel. Circ Res. 1997; 80(3):370–376. [PubMed: 9048657]
8. van Bemmelen MX, Rougier JS, Gavillet B, et al. Cardiac voltage-gated sodium channel Nav1. 5 is regulated by Nedd4-2 mediated ubiquitination. Circ Res. 2004; 95(3):284–291. [PubMed: 15217910]
9. Shy D, Gillet L, Abriel H. Cardiac sodium channel NaV1. 5 distribution in myocytes via interacting proteins: the multiple pool model. Biochim Biophys Acta. 2013; 1833(4):886–894. [PubMed: 23123192]
10. Petitprez S, Zmoos AF, Ogrodnik J, et al. SAP97 and dystrophin macromolecular complexes determine two pools of cardiac sodium channels Nav1. 5 in cardiomyocytes. Circ Res. 2011; 108(3):294–304. [PubMed: 21164104]
11. Maltsev VA, Kyle JW, Mishra S, Undrovinas A. Molecular identity of the late sodium current in adult dog cardiomyocytes identified by Nav1. 5 antisense inhibition. Am J Physiol Heart Circ Physiol. 2008; 295(2):H667–H676. [PubMed: 18552167]
12. London B, Michalec M, Mehdi H, et al. Mutation in glycerol-3-phosphate dehydrogenase 1 like gene (GPD1-L) decreases cardiac Na+ current and causes inherited arrhythmias. Circulation. 2007; 116(20):2260–2268. [PubMed: 17967977]
13. Mazzone A, Strege PR, Tester DJ, et al. A mutation in telethonin alters nav1. 5 function. J Biol Chem. 2008; 283(24):16537–16544. [PubMed: 18408010]
14. Mohler PJ, Rivolta I, Napolitano C, et al. Nav1.5 E1053K mutation causing Brugada syndrome blocks binding to ankyrin-G and expression of Nav1. 5 on the surface of cardiomyocytes. Proc Natl Acad Sci U S A. 2004; 101(50):17533–17538. [PubMed: 15579534]
15. Crotti L, Johnson CN, Graf E, et al. Calmodulin mutations associated with recurrent cardiac arrest in infants. Circulation. 2013; 127(9):1009–1017. [PubMed: 23388215]
16. Bhat HF, Adams ME, Khanday FA. Syntrophin proteins as Santa Claus: role(s) in cell signal transduction. Cell Mol Life Sci. 2013; 70(14):2533–2554. [PubMed: 23263165]
17. Barouch LA, Harrison RW, Skaf MW, et al. Nitric oxide regulates the heart by spatial confinement of nitric oxide synthase isoforms. Nature. 2002; 416(6878):337–339. [PubMed: 11907582]
18. Gavillet B, Rougier JS, Domenighetti AA, et al. Cardiac sodium channel Nav1. 5 is regulated by a multiprotein complex composed of syntrophins and dystrophin. Circ Res. 2006; 99(4):407–414. [PubMed: 16857961]

19. Ueda K, Valdivia C, Medeiros-Domingo A, et al. Syntrophin mutation associated with long QT syndrome through activation of the nNOS-SCN5A macromolecular complex. Proc Natl Acad Sci U S A. 2008; 105(27):9355–9360. [PubMed: 18591664]
20. Williams JC, Armesilla AL, Mohamed TM, et al. The sarcolemmal calcium pump, alpha-1 syntrophin, and neuronal nitric-oxide synthase are parts of a macromolecular protein complex. J Biol Chem. 2006; 281(33):23341–23348. [PubMed: 16735509]
21. Oceandy D, Cartwright EJ, Emerson M, et al. Neuronal nitric oxide synthase signaling in the heart is regulated by the sarcolemmal calcium pump 4b. Circulation. 2007; 115(4):483–492. [PubMed: 17242280]
22. Wu G, Ai T, Kim JJ, et al. alpha-1-syntrophin mutation and the long-QT syndrome: a disease of sodium channel disruption. Circ Arrhythm Electrophysiol. 2008; 1(3):193–201. [PubMed: 19684871]
23. Cheng J, Van Norstrand DW, Medeiros-Domingo A, et al. Alpha1-syntrophin mutations identified in sudden infant death syndrome cause an increase in late cardiac sodium current. Circ Arrhythm Electrophysiol. 2009; 2(6):667–676. [PubMed: 20009079]
24. Hu RM, Tan BH, Orland KM, et al. Digenic inheritance novel mutations in SCN5a and SNTA1 increase late I(Na) contributing to LQT syndrome. Am J Physiol Heart Circ Physiol. 2013; 304(7):H994–H1001. [PubMed: 23376825]
25. Cheng J, Norstrand DW, Medeiros-Domingo A, et al. LQTS-associated mutation A257G in alpha1-syntrophin interacts with the intragenic variant P74L to modify its biophysical phenotype. Cardiogenetics. 2011; 1(1)
26. Balijepalli RC, Kamp TJ. Caveolae, ion channels and cardiac arrhythmias. Prog Biophys Mol Biol. 2008; 98(2–3):149–160. [PubMed: 19351512]
27. Li S, Galbiati F, Volonte D, et al. Mutational analysis of caveolin-induced vesicle formation. Expression of caveolin-1 recruits caveolin-2 to caveolae membranes. FEBS Lett. 1998; 434(1–2): 127–134. [PubMed: 9738464]
28. McNally EM, de Sa ME, Duggan DJ, et al. Caveolin-3 in muscular dystrophy. Hum Mol Genet. 1998; 7(5):871–877. [PubMed: 9536092]
29. Vatta M, Ackerman MJ, Ye B, et al. Mutant caveolin-3 induces persistent late sodium current and is associated with long-QT syndrome. Circulation. 2006; 114(20):2104–2112. [PubMed: 17060380]
30. Cronk LB, Ye B, Kaku T, et al. Novel mechanism for sudden infant death syndrome: Persistent late sodium current secondary to mutations in caveolin-3. Heart Rhythm. 2007; 4(2):161–166. [PubMed: 17275750]
31. Venema VJ, Ju H, Zou R, Venema RC. Interaction of neuronal nitric-oxide synthase with caveolin-3 in skeletal muscle. Identification of a novel caveolin scaffolding/inhibitory domain. J Biol Chem. 1997; 272(45):28187–28190. [PubMed: 9353265]
32. Cheng J, Valdivia CR, Vaidyanathan R, Balijepalli RC, Ackerman MJ, Makielski JC. Caveolin-3 suppresses late sodium current by inhibiting nNOS-dependent S-nitrosylation of SCN5A. J Mol Cell Cardiol. 2013; 61:102–110. [PubMed: 23541953]
33. Vaidyanathan R, Vega AL, Song C, et al. The Interaction of Caveolin 3 with the Inward Rectifier Channel Kir2.1; Physiology and Pathology related to LQT9. J Biol Chem. 2013
34. Ou X, Ji C, Han X, et al. Crystal structures of human glycerol 3-phosphate dehydrogenase 1 (GPD1). J Mol Biol. 2006; 357(3):858–869. [PubMed: 16460752]
35. Weiss R, Barmada MM, Nguyen T, et al. Clinical and molecular heterogeneity in the Brugada syndrome: a novel gene locus on chromosome 3. Circulation. 2002; 105(6):707–713. [PubMed: 11839626]
36. Van Norstrand DW, Valdivia CR, Tester DJ, et al. Molecular and Functional Characterization of Novel Glycerol-3-Phosphate Dehydrogenase 1 Like Gene (GPD1-L) Mutations in Sudden Infant Death Syndrome. Circulation. 2007
37. Valdivia CR, Ueda K, Ackerman MJ, Makielski JC. GPD1L links redox state to cardiac excitability by PKC-dependent phosphorylation of the sodium channel SCN5A. Am J Physiol Heart Circ Physiol. 2009; 297(4):H1446–H1452. [PubMed: 19666841]

38. Liu M, Sanyal S, Gao G, et al. Cardiac Na+ current regulation by pyridine nucleotides. Circ Res. 2009; 105(8):737–745. [PubMed: 19745168]
39. Liu M, Liu H, Dudley SC Jr. Reactive oxygen species originating from mitochondria regulate the cardiac sodium channel. Circ Res. 2010; 107(8):967–974. [PubMed: 20724705]
40. Makiyama T, Akao M, Haruna Y, et al. Mutation analysis of the glycerol-3 phosphate dehydrogenase-1 like (GPD1L) gene in Japanese patients with Brugada syndrome. Circ J. 2008; 72(10):1705–1706. [PubMed: 18762705]
41. Westaway SK, Reinier K, Huertas-Vazquez A, et al. Common variants in CASQ2, GPD1L, and NOS1AP are significantly associated with risk of sudden death in patients with coronary artery disease. Circ Cardiovasc Genet. 2011; 4(4):397–402. [PubMed: 21685173]
42. Steggerda SM, Paschal BM. Identification of a conserved loop in Mog1 that releases GTP from Ran. Traffic. 2001; 2(11):804–811. [PubMed: 11733047]
43. Marfatia KA, Harreman MT, Fanara P, Vertino PM, Corbett AH. Identification and characterization of the human MOG1 gene. Gene. 2001; 266(1–2):45–56. [PubMed: 11290418]
44. Wu L, Yong SL, Fan C, et al. Identification of a new co-factor, MOG1, required for the full function of cardiac sodium channel Nav 1. 5. J Biol Chem. 2008; 283(11):6968–6978. [PubMed: 18184654]
45. Chakrabarti S, Wu X, Yang Z, et al. MOG1 rescues defective trafficking of Na(v)1. 5 mutations in Brugada syndrome and sick sinus syndrome. Circ Arrhythm Electrophysiol. 2013; 6(2):392–401. [PubMed: 23420830]
46. Kattygnarath D, Maugenre S, Neyroud N, et al. MOG1: a new susceptibility gene for Brugada syndrome. Circ Cardiovasc Genet. 2011; 4(3):261–268. [PubMed: 21447824]
47. Olesen MS, Jensen NF, Holst AG, et al. A novel nonsense variant in Nav1. 5 cofactor MOG1 eliminates its sodium current increasing effect and may increase the risk of arrhythmias. Can J Cardiol. 2011; 27(4):523. [PubMed: 21621375]
48. Campuzano O, Berne P, Selga E, et al. Brugada syndrome and p.E61X_RANGRF. Cardiol J. 2014
49. Bass-Zubek AE, Godsel LM, Delmar M, Green KJ. Plakophilins: multifunctional scaffolds for adhesion and signaling. Curr Opin Cell Biol. 2009; 21(5):708–716. [PubMed: 19674883]
50. Kowalczyk AP, Green KJ. Structure, function, and regulation of desmosomes. Prog Mol Biol Transl Sci. 2013; 116:95–118. [PubMed: 23481192]
51. van Tintelen JP, Entius MM, Bhuiyan ZA, et al. Plakophilin-2 mutations are the major determinant of familial arrhythmogenic right ventricular dysplasia/cardiomyopathy. Circulation. 2006; 113(13):1650–1658. [PubMed: 16567567]
52. Valdivia CR, Chu WW, Pu JL, et al. Increased late sodium current in myocytes from a canine heart failure model and from failing human heart. J Mol Cell Cardiol. 2005; 38(3):475–483. [PubMed: 15733907]
53. Noorman M, Hakim S, Kessler E, et al. Remodeling of the cardiac sodium channel, connexin43, and plakoglobin at the intercalated disk in patients with arrhythmogenic cardiomyopathy. Heart Rhythm. 2013; 10(3):412–419. [PubMed: 23178689]
54. Sato PY, Musa H, Coombs W, et al. Loss of plakophilin-2 expression leads to decreased sodium current and slower conduction velocity in cultured cardiac myocytes. Circ Res. 2009; 105(6):523–526. [PubMed: 19661460]
55. Cerrone M, Noorman M, Lin X, et al. Sodium current deficit and arrhythmogenesis in a murine model of plakophilin-2 haploinsufficiency. Cardiovasc Res. 2012; 95(4):460–468. [PubMed: 22764151]
56. Cerrone M, Lin X, Zhang M, et al. Missense mutations in plakophilin-2 cause sodium current deficit and associate with a brugada syndrome phenotype. Circulation. 2014; 129(10):1092–1103. [PubMed: 24352520]
57. Cerrone M, Delmar M. Desmosomes and the sodium channel complex: Implications for arrhythmogenic cardiomyopathy and Brugada syndrome. Trends Cardiovasc Med. 2014
58. Wielowieyski PA, Sevinc S, Guzzo R, Salih M, Wigle JT, Tuana BS. Alternative splicing, expression, and genomic structure of the 3′ region of the gene encoding the sarcolemmal-associated proteins (SLAPs) defines a novel class of coiled-coil tail-anchored membrane proteins. J Biol Chem. 2000; 275(49):38474–38481. [PubMed: 10986292]

59. Ishikawa T, Sato A, Marcou CA, et al. A novel disease gene for Brugada syndrome: sarcolemmal membrane-associated protein gene mutations impair intracellular trafficking of hNav1. 5. Circ Arrhythm Electrophysiol. 2012; 5(6):1098–1107. [PubMed: 23064965]
60. te Velthuis AJ, Isogai T, Gerrits L, Bagowski CP. Insights into the molecular evolution of the PDZ/LIM family and identification of a novel conserved protein motif. PLoS One. 2007; 2(2):e189. [PubMed: 17285143]
61. Vatta M, Mohapatra B, Jimenez S, et al. Mutations in Cypher/ZASP in patients with dilated cardiomyopathy and left ventricular non-compaction. J Am Coll Cardiol. 2003; 42(11):2014–2027. [PubMed: 14662268]
62. Zheng M, Cheng H, Banerjee I, Chen J. ALP/Enigma PDZ-LIM domain proteins in the heart. J Mol Cell Biol. 2010; 2(2):96–102. [PubMed: 20042479]
63. Xi Y, Ai T, De Lange E, et al. Loss of function of hNav1. 5 by a ZASP1 mutation associated with intraventricular conduction disturbances in left ventricular noncompaction. Circ Arrhythm Electrophysiol. 2012; 5(5):1017–1026. [PubMed: 22929165]
64. Allouis M, Le Bouffant F, Wilders R, et al. 14-3-3 is a regulator of the cardiac voltage-gated sodium channel Nav1. 5. Circ Res. 2006; 98(12):1538–1546. [PubMed: 16728661]
65. Ziane R, Huang H, Moghadaszadeh B, Beggs AH, Levesque G, Chahine M. Cell membrane expression of cardiac sodium channel Na(v)1. 5 is modulated by alpha-actinin-2 interaction. Biochemistry. 2010; 49(1):166–178. [PubMed: 19943616]
66. Mohler PJ, Wehrens XH. Mechanisms of human arrhythmia syndromes: abnormal cardiac macromolecular interactions. Physiology (Bethesda). 2007; 22:342–350. [PubMed: 17928548]
67. Lowe JS, Palygin O, Bhasin N, et al. Voltage-gated Nav channel targeting in the heart requires an ankyrin-G dependent cellular pathway. J Cell Biol. 2008; 180(1):173–186. [PubMed: 18180363]
68. Dhar MJ, Chen C, Rivolta I, et al. Characterization of sodium channel alpha- and beta-subunits in rat and mouse cardiac myocytes. Circulation. 2001; 103(9):1303–1310. [PubMed: 11238277]
69. Valdivia CR, Nagatomo T, Makielski JC. Late currents affect kinetics for heart and skeletal Na channel α and β1 subunits expressed in HEK293 cells. Journal of Molecular & Cellular Cardiology. 2002 In press.
70. Maltsev VA, Kyle JW, Undrovinas A. Late Na+ current produced by human cardiac Na+ channel isoform Nav1. 5 is modulated by its beta1 subunit. J Physiol Sci. 2009; 59(3):217–225. [PubMed: 19340536]
71. Herfst LJ, Potet F, Bezzina CR, et al. Na+ channel mutation leading to loss of function and non-progressive cardiac conduction defects. J Mol Cell Cardiol. 2003; 35(5):549–557. [PubMed: 12738236]
72. Nuss HB, Chiamvimonvat N, Perez-Garcia MT, Tomaselli GF, Marban E. Functional association of the β1 subunit with human cardiac (hH1) and rat skeletal muscle (μ1) sodium channel α subunits expressed in Xenopus oocytes. J Gen Physiol. 1995; 106(6):1171–1191. [PubMed: 8786355]
73. Lopez-Santiago LF, Meadows LS, Ernst SJ, et al. Sodium channel Scn1b null mice exhibit prolonged QT and RR intervals. J Mol Cell Cardiol. 2007
74. Malhotra JD, Chen C, Rivolta I, et al. Characterization of sodium channel α- and β-Subunits in rat and mouse cardiac myocytes. Circulation. 2001; 103(9):1303–1310. [PubMed: 11238277]
75. Johnson D, Bennett ES. Isoform-specific effects of the beta2 subunit on voltage-gated sodium channel gating. J Biol Chem. 2006; 281(36):25875–25881. [PubMed: 16847056]
76. Mishra S, Undrovinas NA, Maltsev VA, Reznikov V, Sabbah HN, Undrovinas A. Post-transcriptional silencing of SCN1B and SCN2B genes modulates late sodium current in cardiac myocytes from normal dogs and dogs with chronic heart failure. Am J Physiol Heart Circ Physiol. 2011; 301(4):H1596–H1605. [PubMed: 21705762]
77. Hakim P, Brice N, Thresher R, et al. Scn3b knockout mice exhibit abnormal sino-atrial and cardiac conduction properties. Acta Physiol (Oxf). 2010; 198(1):47–59. [PubMed: 19796257]
78. Fahmi AI, Patel M, Stevens EB, et al. The sodium channel beta-subunit SCN3b modulates the kinetics of SCN5a and is expressed heterogeneously in sheep heart. J Physiol. 2001; 537(Pt 3): 693–700. [PubMed: 11744748]

79. Hakim P, Gurung IS, Pedersen TH, et al. Scn3b knockout mice exhibit abnormal ventricular electrophysiological properties. Prog Biophys Mol Biol. 2008; 98(2–3):251–266. [PubMed: 19351516]
80. Medeiros-Domingo A, Kaku T, Tester DJ, et al. SCN4B-Encoded Sodium Channel {beta}4 Subunit in Congenital Long-QT Syndrome. Circulation. 2007; 116:136–142.
81. Remme CA, Scicluna BP, Verkerk AO, et al. Genetically determined differences in sodium current characteristics modulate conduction disease severity in mice with cardiac sodium channelopathy. Circ Res. 2009; 104(11):1283–1292. [PubMed: 19407241]
82. Hund TJ, Koval OM, Li J, et al. A beta(IV)-spectrin/CaMKII signaling complex is essential for membrane excitability in mice. J Clin Invest. 2010; 120(10):3508–3519. [PubMed: 20877009]
83. Kim J, Ghosh S, Liu H, Tateyama M, Kass RS, Pitt GS. Calmodulin mediates Ca2+ sensitivity of sodium channels. J Biol Chem. 2004; 279(43):45004–45012. [PubMed: 15316014]
84. Chagot B, Chazin WJ. Solution NMR structure of Apo-calmodulin in complex with the IQ motif of human cardiac sodium channel NaV1. 5. J Mol Biol. 2011; 406(1):106–119. [PubMed: 21167176]
85. Tan HL, Kupershmidt S, Zhang R, et al. A calcium sensor in the sodium channel modulates cardiac excitability. Nature. 2002; 415(6870):442–447. [PubMed: 11807557]
86. Aiba T, Hesketh GG, Liu T, et al. Na+ channel regulation by Ca2+/calmodulin and Ca2+/calmodulin-dependent protein kinase II in guinea-pig ventricular myocytes. Cardiovasc Res. 2010; 85(3):454–463. [PubMed: 19797425]
87. Koval OM, Snyder JS, Wolf RM, et al. Ca2+/Calmodulin-Dependent Protein Kinase II-Based Regulation of Voltage-Gated Na+ Channel in Cardiac Disease. Circulation. 2012; 126(17):2084–2094. [PubMed: 23008441]
88. van der Velden HM, Jongsma HJ. Cardiac gap junctions and connexins: their role in atrial fibrillation and potential as therapeutic targets. Cardiovasc Res. 2002; 54(2):270–279. [PubMed: 12062332]
89. Malhotra JD, Thyagarajan V, Chen C, Isom LL. Tyrosine-phosphorylated and nonphosphorylated sodium channel beta1 subunits are differentially localized in cardiac myocytes. J Biol Chem. 2004; 279(39):40748–40754. [PubMed: 15272007]
90. Jansen JA, Noorman M, Musa H, et al. Reduced heterogeneous expression of Cx43 results in decreased Nav1. 5 expression and reduced sodium current that accounts for arrhythmia vulnerability in conditional Cx43 knockout mice. Heart Rhythm. 2012; 9(4):600–607. [PubMed: 22100711]
91. Rizzo S, Lodder EM, Verkerk AO, et al. Intercalated disc abnormalities, reduced Na(+) current density, and conduction slowing in desmoglein-2 mutant mice prior to cardiomyopathic changes. Cardiovasc Res. 2012; 95(4):409–418. [PubMed: 22764152]
92. Albesa M, Ogrodnik J, Rougier JS, Abriel H. Regulation of the cardiac sodium channel Nav1. 5 by utrophin in dystrophin-deficient mice. Cardiovasc Res. 2011; 89(2):320–328. [PubMed: 20952415]
93. Wang C, Hennessey JA, Kirkton RD, et al. Fibroblast growth factor homologous factor 13 regulates Na+ channels and conduction velocity in murine hearts. Circ Res. 2011; 109(7):775–782. [PubMed: 21817159]
94. Ou YJ, Strege P, Miller SM, et al. Syntrophin gamma 2 regulates SCN5A Gating by a PDZ domain-mediated interaction. J Biol Chem. 2003; 278(3):1915–1923. [PubMed: 12429735]
95. Jespersen T, Gavillet B, van Bemmelen MX, et al. Cardiac sodium channel Na(v)1. 5 interacts with and is regulated by the protein tyrosine phosphatase PTPH1. Biochem Biophys Res Commun. 2006; 348(4):1455–1462. [PubMed: 16930557]
96. Van Norstrand DW, Valdivia CR, Tester DJ, et al. Molecular and functional characterization of novel glycerol-3-phosphate dehydrogenase 1 like gene (GPD1-L) mutations in sudden infant death syndrome. Circulation. 2007; 116(20):2253–2259. [PubMed: 17967976]
97. Trane AE, Pavlov D, Sharma A, et al. Deciphering the binding of caveolin-1 to client protein endothelial nitric oxide synthase (eNOS): scaffolding sub-domain identification, interaction modeling, and biological significance. J Biol Chem. 2014

Key points

- Sodium current, which underlies cardiac excitability, flows through a pore protein $Na_V1.5$ which is part of a larger complex of interacting proteins
- Mutations in 7 $Na_V1.5$ interacting proteins have been associated with dysfunctional sodium current and inherited cardiac diseases.
- The mechanisms by which mutations in interacting proteins cause specific dysfunction involve targeting/trafficking and phosphorylation/nitrosylation of the $Na_V1.5$ complex.
- Mutations in as yet unidentified interacting proteins may account for cardiac disease for which a genetic basis is not yet known

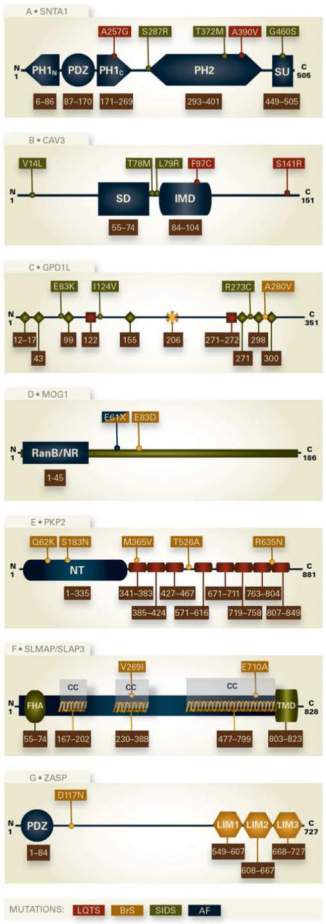

Figure 1.

Diagrams of SCIPs with locations of mutations causing $Na_V1.5$-related cardiac disease and select functional domains. The N- and C-termini are indicated as N and C with the amino acid number beneath. The amino acids corresponding to each domain are shown below (amino acid range for domains are assigned based on http://www.uniprot.org/). Disease-causing mutations are indicated at the top of each panel with the box color coded for diseases long QT syndrome (LQTS), Brugada syndrome (BrS), sudden infant death syndrome (SIDS), and atrial fibrillation (AF) as noted at the figure bottom, with references for these mutations given in Table 2. A. SNTA1: Two parts of the split plextrin homology domain are shown as $PH1_N$ and $PH1_C$ with the PDZ domain located between them. PH2 is the second plextrin homology domain and SU is the syntrophin unique domain. http://www.uniprot.org/uniprot/Q13424 B. CAV3: SD is the CAV3 scaffold domain and IMD is the intramembrane domain. http://www.uniprot.org/uniprot/P56539 C. GPD1L: NAD binding sites are indicated as diamonds, substrate binding regions are red boxes and the active site is indicated as *. http://www.uniprot.org/uniprot/Q8N335 D. MOG1: RanBD/NR is the Ran binding/GTP release domain[42] http://www.uniprot.org/uniprot/Q9HD47 E. PKP2: The N-terminal domain is marked NT and the eight armadillo repeats are indicated as red boxes. http://www.uniprot.org/uniprot/Q13835 F. SLMAP: The long cytoplasmic N-terminus with a forkhead-associated domain is marked FHA, coiled-coiled leucine zipper

domains labeled CC are shown as coils, and a transmembrane domain is labeled TMD. http://www.uniprot.org/uniprot/Q14BN4 G. ZASP: The PDZ domain at the N-terminal end is marked PDZ and the 3 Lim zinc binding domains labeled Lim1, Lim2 and Lim3. http://www.uniprot.org/uniprot/O75112

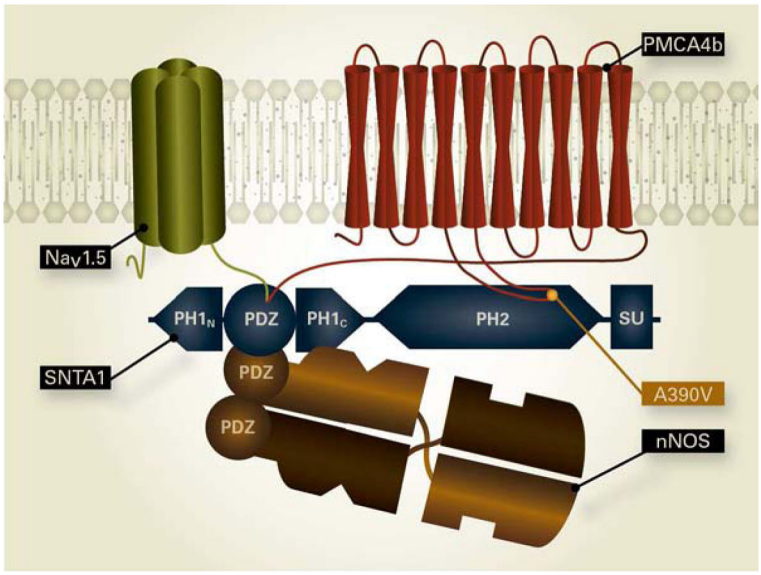

Figure 2.

Cartoon of the Na$_V$1.5/SNTA1/nNOS/PMCA4b complex. The PDZ binding domain at the C-terminus of Na$_V$1.5 binds to the PDZ domain of SNTA1. One of the PDZ domains from the nNOS dimer associates with the PDZ domain of SNTA1. PMCA4b interacts with SNTA1 at two locations, the 4–5 cytoplasmic loop binds to the PH2/SU region of SNTA1 and the PDZ binding domain in the C-terminus of PMCA4b can also associate with PDZ in SNTA1. The A390V-SNTA1 BrS mutation that disrupts binding of PMCA4b to SNTA1 is shown. The release of PMCA4b from the complex results in upregulation of nNOS and increased nitrosylation of Na$_V$1.5 and increased late I_{Na}.

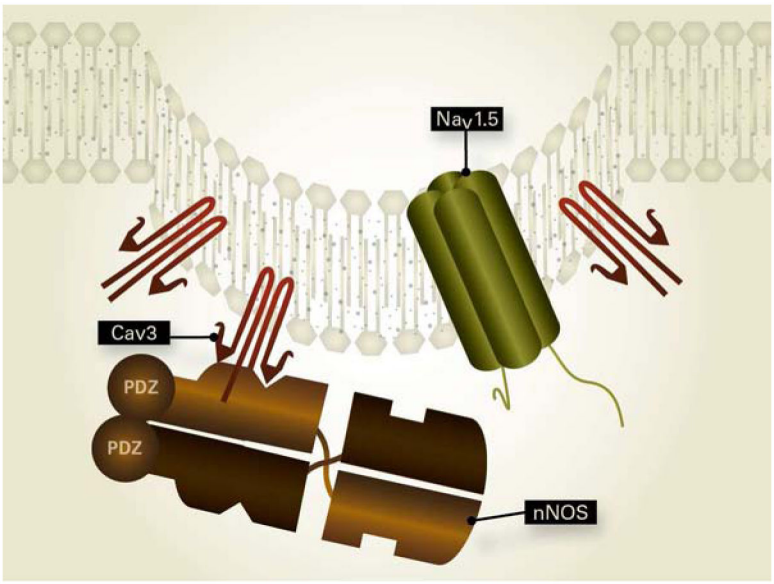

Figure 3.

The CAV3/nNOS/Na$_V$1.5 complex in caveolae. The triangles on CAV3 represents amino acids 62–72 and are homologous to the CAV1 region that has been shown to bind to and inhibit eNOS activity[97]. The binding site on nNOS shown is located at W681 and W683 and is based on the homologous site on eNOS[97].

Table 1

$Na_V1.5$ interacting proteins (SCIPs)

Protein	Gene	Size (aa)	Evidence for $Na_V1.5$ Association	Effect on I_{Na}
14-3-3η	*Ywhah*	246	Co-IP[64]	(↓)RecR, (−) SSI[64]
α-Actinin 2	*Actn2*	894	Co-IP[65]	(↑) I_{Na} Peak[65]
Ankyrin G	*Ank3*	4377	Co-IP[66, 67]	(↑)Surface Density, (↑)I_{Na} Peak[66, 67]
α1-Syntrophin	*Snta1*	505	GST-PD, Co-IP[18, 19, 23]	Regulate I_{Na} Late [18, 19, 23]
β1, β1b	*Scn1b*	β1 218 β1b 268	Co-IP[68]	Kinetics conflicting (↓) I_{Na} Late[69, 70] (↑)RecR[71] (↑)I_{Na} Peak[72, 73]
β2	*Scn2b*	215	Co-IP[74]	Sialylation Status[75], (↑)Late Current[76]
β3	*Scn3b*	215	Co-IP[77]	(↑)I_{Na}, (↑)RecR[78](+) SSI, (↑)RefractPer.,[79]
β4	*Scn4b*	228	Co-IP[80]	(↑) AP Upstroke Velocity, (+) SSI[81]
β4 Spectrin	*Sptbn4*	2,564	Co-IP[82]	(↑)I_{Na} Peak, (+) SSI via Phos.@ S571[82]
Calmodulin	*Calm1*	152	GST-PD[83] NMR[84]	(+)SSI[85, 86]
CaMKinase IIδ isoform 3	*Camk2d*	510	Co-IP[82]	(−) SSI, Phos. @ S571S, 516, T594 (↑) I_{Na} Late[87]
Caveolin-3	*Cav3*	151	Co-IP[32]	(↓) I_{Na} Late[29, 30, 32]
Connexin 40	*Gja5*	358	Co-loc[88]	?
Connexin 43	*Gja1*	382	Co-IP[89]	(↑) I_{Na} Peak[90]
Desmoglein 2	*Dsg2*	1118	Co-IP[91]	(↑) I_{Na} Peak[91]
Dystrophin	*Dmd*	3,685	Co-IP[92]	(↑)I_{Na} Peak[92]
Fibroblast growth factor FGF12B, 13	*Fgf12* *Fgf13*	181	Co-IP[93]	(+) SSI, (↑) RecR[93]
λ2 syntrophin	*Sntg2*	539	GST-PD[94]	(+) Activ Ou, 2003 175/id}
GPD1L	*Gpd1l*	351	GST-PD[37]	(↑)I_{Na} by phosphorylation[37] 80 or ROS[39]
MOG1	*Rangrf*	218	GST-PD, co-IP[44]	(↑)Surface Density, (↑)I_{Na} Peak[44]
Nedd4-2	*Nedd4l*	975	GST-PD[8]	(↑) I_{Na} Peak[8]
nNOS	*Nos1*	1434	GST-PD, Co-IP[19]	(↑)I_{Na} Late[19] (↑) I_{Na} Peak[22, 23]
Plakophilin-2	*Pkp2*	881	Co-IP[54]	(↑) I_{Na} Peak, (+) SSI[56]
PMCA4b	*Atp2b4*	1241	GST-PD, Co-IP[19, 20]	(↓) I_{Na} Late[19]
SAP97	*Dlg1*	904	Co-IP[10]	(↑) I_{Na} Peak[10]
SLMAP	*Slmap*	828	*[59]	(↑) I_{Na} Peak[59]
Telethonin	*Tcap*	167	Co-IP[13]	(−) Activ[13]
PTPH1	*Ptpn3*	913	GST-PD[95]	(−) SSI[95]
Utrophin	*Utrn*	3433	Co-IP[92]	(↓) I_{Na} Peak[92]

Protein	Gene	Size (aa)	Evidence for $Na_V1.5$ Association	Effect on I_{Na}
Zasp	*Ldb3*	727	GST-PD[63]	(↑) I_{Na} Peak [63]

Adapted from Adsit GS, Vaidyanathan R, Galler CM, Kyle JW, Makielski JC. Channelopathies from mutations in the cardiac sodium channel protein complex. J Mol Cell Cardiol 2013.

*SLMAP has not yet been shown to associate with NaV1.5.

Abbreviations: Co-IP = co-immunoprecipitation; GST-PD = pull-down with a GST fusion protein; (↓) = decrease in; (↑) = increase in; (+) = depolarizing shift; (−) = hyperpolarizing shift; Activ.= activation; RecR = recovery rate; RefractPer.=refractory period; SSI=steady state inactivation; INa Peak= peak sodium current; INa Late =late sodium current or persistent current

Table 2

Sodium channel interacting protein (SCIP) with disease-causing mutations that have been shown to affect I_{Na}

SCIP	Gene name	Common Name	Chromosome	MW kDa	Splice Variants	Disease	Disease mutations
SNTA1	*SNTA1*	α1-Syntrophin	20q11.2	54	1	LQT12[19, 25] SIDS[23]	A257G, A261V, 390V T262P, T372M, G460S
CAV3	*CAV3*	Caveolin-3	3p25	17	1	LQT9[29] SIDS[30]	A85T, F97C, S141R V14L, T78M, L79R
GPD11	*GPD1L*	Glycerol -3- Phoshphate deydrogenase 1-Like	3p22.3	38	1	BrS[12, 38] SIDS[37, 96]	A280V E83K, I124V, R273C
MOG1	*RANGRF*	MOG1	17p13.1	20	4	BrS[46] AF[47]	E83D E61X
PKP2	*PKP2*	Plakophillin-2	12p11	97	2	BrS[56]	S183N, M365V, R635Q T526A
ZASP	*LDB3*	Z-band alternatively spliced PDZ motif protein	10q23.2	77	7	LVNC[61, 63]	D117N (isoform2)
SLMAP	*SLMAP*	Sarcolemma Membrane- Associated Protein	3p21.2	95	8	BrS[59]	V269I, E710A
$Na_V1.5$	*SCN5A*	Cardiac Sodium Channel	3p21	227	6	LQT3, BrS, SIDS, CM, and others	>400

LQT, long QT; SIDS, sudden infant death syndrome; BrS, Brugada syndrome; AF, atrial fibrillation; LVNC, left ventricular non-compaction; CM, cardiomyopathy. Underlined mutations reported to be associated with more than one syndrome. Information on splice variants can be found at the Uniprot web site, specifically for SNTA1 http://www.uniprot.org/uniprot/Q13424, CAV3 http://www.uniprot.org/uniprot/P56539, GPD1L http://www.uniprot.org/uniprot/Q8N335 MOG1 http://www.uniprot.org/uniprot/Q9HD47 PKP2 http://www.uniprot.org/uniprot/Q13835 SLMAP http://www.uniprot.org/uniprot/Q14BN4 ZASP http://www.uniprot.org/uniprot/O75112

Card Electrophysiol Clin. Author manuscript; available in PMC 2015 December 01.