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Sodium channel β subunits: emerging targets in channelopathies

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Abstract

Voltage-gated sodium channels (VGSCs) are responsible for initiation and propagation of action potentials in excitable cells. VGSCs in mammalian brain are heterotrimeric complexes of α and β subunits. Originally called "auxiliary," we now know that β subunit proteins are multifunctional signaling molecules that play roles in both excitable and non-excitable cell types, and with or without the pore-forming α subunit present. β subunits function in VGSC and potassium channel modulation, cell adhesion, and gene regulation, with particularly important roles in brain development. Mutations in the genes encoding β subunits are linked to a number of diseases, including epilepsy, sudden death syndromes like SUDEP and SIDS, and cardiac arrhythmia. While VGSC β subunit-specific drugs have not yet been developed, this protein family is an emerging therapeutic target.

Keywords

cell adhesion; development; epilepsy; arrhythmia; pain; neurodegenerative disease

I. Introduction

Voltage-gated sodium channels (VGSCs) are responsible for generation of the rising phase and propagation of the action potential (AP) in excitable cells, including neurons and cardiac myocytes (1). VGSCs have been purified as heterotrimeric complexes of α and β subunits from mammalian brain where a central α subunit forms the ion-conducting pore and is complexed with two different β subunits (2). This is postulated, but not proven, to be the case in other excitable tissues. Originally characterized as "auxiliary," important only in modulation of VGSC function, the β subunit proteins are now known to be dynamic, multifunctional molecules that engage in diverse and essential roles throughout multiple tissues and systems, in both excitable and non-excitable cell types, and with or without VGSC α subunits (3). Their ability to participate in both conducting and non-conducting roles makes VGSC β subunits unique among voltage-gated ion channel subunits. During the more than two decades since the first VGSC β subunits were identified, a growing body of research has identified the importance of these proteins not only in normal physiology, but also in numerous pathophysiologies including channelopathies.

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The breadth of β subunit function hinges on the key structural and functional motif common to all members of this family of proteins: an immunoglobulin (Ig) loop enabling them to function as cell adhesion molecules (CAMs) (3; 4). These adhesive functions are critical to brain development and thus to excitability, including the processes of neurite outgrowth, axon pathfinding, fasciculation, and cell migration, and are postulated to play similar roles in heart and peripheral nerve (3; 4). In their roles as ion channel modulators, not only of VGSCs but also voltage-gated K^+ channels (VGKCs) (5–7), β subunits make important contributions to the regulation of neuronal firing. As substrates for sequential cleavage by β- (BACE) and γ-secretases, β subunits contribute to not only cell adhesion but also to the regulation of VGSC α subunit gene expression (8). Taking all of these roles into consideration, it is no wonder that mutations in the genes encoding VGSC β subunits are linked to pathophysiology. This review highlights the primary disease areas in which VGSC β subunit gene mutations are linked as well as some of the novel pathways and disorders with which β subunits are only now beginning to be associated, with the advent of powerful tools such as genome-wide association studies (GWAS) and whole exome sequencing.

II. Fundamentals: β Subunits 101

1. VGSC Genes and Structure

Nine VGSC α subunits have been identified, denoted $Na_v1.1$-$Na_v1.9$ and encoded by a gene family with nomenclature *SCN(X)A* (9). Sodium current (I_{Na}) flow down its concentration gradient occurs in response to changes in membrane potential, wherein VGSCs open, or activate, in response to a depolarizing stimulus, then inactivate via closure of an intracellular inactivation gate, becoming refractory to conduction until the channel reaches the closed state and is thus able to respond to another stimulus (1). VGSC α subunits are categorized into two groups based on their sensitivity to tetrodotoxin (TTX). TTX-sensitive (TTX-S) α subunits (Na_v 1.1, $Na_v1.2$, $Na_v1.3$, $Na_v1.4$, $Na_v1.6$, and $Na_v1.7$) are blocked by nanomolar concentrations of TTX, whereas TTX-resistant (TTX-R) α subunits are blocked by micromolar concentrations of TTX (9).

The five VGSC β subunit proteins are encoded by a family of four genes. β1 and its splice variant β1B are encoded by *SCN1B* (10–13), and β2, β3, and β4 are encoded by *SCN2B* (14), *SCN3B* (15), and *SCN4A* (16), respectively. All five VGSC β subunits contain an extracellular Ig domain homologous to V-type Ig loop motifs present in the Ig superfamily (IgSF) of CAMs (11; 15–18), an observation that provided key early evidence of the multifunctionality of these proteins. With the exception of β1B, β subunits have type 1 topology, consisting of a single polypeptide chain with an extracellular N-terminus, a single transmembrane-spanning segment, and an intracellular C-terminus. In the case of β1B (originally called β1A), normal splicing of the exon 3/intron 3 boundary does not occur, leading to in-frame retention of intron 3 and generation of an alternate C-terminal sequence that does not include a transmembrane domain (11). As a result, β1B is a developmentally regulated secreted protein (12).

VGSC α and β subunits interact via two distinct mechanisms. β1 and β3, which share 57% sequence homology (15), interact non-covalently with α subunits via their N- and C-termini (18–20). Although β1B is a secreted protein, it selectively associates with $Na_v1.5$, but not

with $Na_v1.1$ or $Na_v1.3$, in heterologous systems (12). β2 and β4 share 35% sequence homology (16) and engage in covalent interactions with α subunits via a single N-terminal cysteine in the extracellular Ig loop (2; 16; 21): Cys-26 in β2 (22) or Cys-58 in β4 (23).

Until recently, β subunits were modelled on the known structure of the extracellular domain of the IgSF CAM myelin P0, with which β subunits, especially β1 and β3, share a high degree of sequence homology (14; 15; 18). Structures for the β3 and β4 extracellular domains have now been published, confirming elements that had been deduced from the myelin P0 structure (23; 24) and providing useful and more directly comparable models for β1 and β2. Two cysteine residues in the extracellular domain of each β subunit are responsible for formation and maintenance of the Ig-fold and its multiple constituent β-sheets, and their positions are conserved across all five β subunits. For β3, a second intramolecular disulfide bond provides further stabilization. Interestingly, β3 associates with $Na_v1.5$ in a trimeric complex that is capable of forming oligomers, rather than the canonical heterotrimeric complex of two non-identical β subunits with a single α subunit (24). The residues mediating this trimeric assembly are unique to β3 and not predicted to occur in β1 (24), supporting the distinct expression patterns and postulated functional roles of these proteins (reviewed in (4)). Thus, the canonical heterotrimeric VGSC structure, based on channel purification from brain, may not hold across tissues.

2. Expression and Localization

VGSC β subunits are expressed in a wide range of tissue and cell types, including excitable and non-excitable cells, and their expression patterns vary with development. VGSC α subunits are found not only in mammals but have orthologs in invertebrates, bacteria and electric eel, among others (4). β subunit proteins are expressed in a wide range of vertebrate species, but not invertebrates, suggesting that their evolution followed that of the pore-forming α subunit. In support of this, *Drosophila* VGSC α subunits associate with a non-pore forming subunit, tipE, that is not homologous to vertebrate β subunits but does modulate channel function, suggesting a separate evolutionary pathway (25). In mammalian brain, β1 and β2 predominate in postnatal development with peak levels in adult (10; 14), while expression of β1B and β3 are higher in embryonic development and early life (11; 12; 26). This developmental expression pattern is different in heart, where β1B and β3 expression persist into adulthood (11; 26). Details about the developmental profile of β4 expression are not yet known. In general, β subunit expression patterns are distinct and, in some locations, complementary, suggesting that each β subunit plays a unique non-compensatory role. Table I summarizes the current understanding of β subunit expression in selected cell types in vertebrate nervous system and cardiac tissue.

β subunits are localized to a number of important subcellular compartments in which they are components of macromolecular complexes, enabling them to engage in specialized functions. In brain and peripheral nerve, these include the axon initial segment (AIS) and nodes of Ranvier where VGSC α subunits are clustered at high density (21; 27–31) (Table 1). At both AIS and nodes, C-terminal association with the adaptor protein ankyrin-G is postulated to anchor β subunits to the cytoskeletal protein βIV spectrin (32). The specific components of VGSC macromolecular complexes differ based on their specific subcellular

location. For example, at nodes of Ranvier, β subunits can associate with α subunits tightly clustered within the nodal gap and are thus positioned for channel modulation during rapid saltatory conduction (21; 27–29). In the paranodal subcompartment adjacent to the nodal gap, β1 is able to engage in adhesive functions that contribute to the close apposition of axo-glial membranes; here, β1 interacts with a trimeric complex of axo-glial paranodal proteins consisting of axonal contactin and Caspr and glial neurofascin-155 (Nf-155) (33; 34). β1 localization to this subcellular compartment is postulated to contribute to the establishment and maintenance of paranodes and formation of the nodal gap.

3. Post-Translational Modification

VGSC β subunit function, localization, and expression are regulated by multiple post-translational modifications, including phosphorylation, glycosylation, and proteolytic cleavage (reviewed in (4)). The β1 intracellular domain (ICD) contains a tyrosine residue at position 181 that is a substrate for phosphorylation (32; 35), likely through the src family of kinases, particularly fyn kinase (36; 37). In vitro, phosphorylation of β1-Y181 is essential for β1-mediated recruitment of ankyrin to points of cell-cell contact; mutation of this site abolishes ankyrin recruitment without abolishing channel modulatory function (32). Phosphorylation also plays a role in β subunit trafficking to the correct subcellular compartments, as evidenced by differential localization of phosphorylated and non-phosphorylated β1 in heart (35). β subunit proteins are highly glycosylated, containing 3–4 N-linked glycosylation sites (10; 18; 38), which accounts for approximately 1/3 of the molecular weight of the mature proteins. These modifications contribute to both surface expression and channel modulation (38). β subunits are also substrates for proteolytic cleavage by BACE1 and γ-secretase, sequentially shedding first the extracellular domain, resulting in generation of a soluble Ig loop-containing protein similar to β1B (12) or an engineered soluble Fc fusion protein (39; 40), and then releasing the shorter ICD (8). These cleavage events may have important functional roles: γ-secretase cleavage is required for β1-mediated neurite outgrowth in vitro (3), and γ-secretase cleavage of β2 in vitro leads to translocation of the ICD to the nucleus where it increases *SCN1A* expression (41).

4. β Subunits in Channel Modulation

Canonically, VGSC β subunits function in concert with α subunits to promote channel trafficking to the plasma membrane and to modulate VGSC biophysical properties. Co-expression of β subunits in vitro and in vivo leads to increased α subunit expression at the plasma membrane. During VGSC biosynthesis, the final step before plasma membrane insertion is covalent association of α with β2 (42), such that hippocampal neurons from *Scn2b* null mice show ~50% reductions in I_{Na} density and in the number of α subunits at the plasma membrane (27). Deletion of β subunits can also lead to alterations in the complement of α subunits expressed in specific brain regions; in *Scn1b* null hippocampus, CA3 neurons display decreased levels of $Na_v1.1$ and increased levels of $Na_v1.3$ (28). Additionally, these interactions display functional reciprocity: in *Scn1b* null cerebellar granule neurons (CGNs), AIS expression of $Na_v1.6$ is reduced in favor of increased expression of $Na_v1.1$ and $Na_v1.2$, and conversely, expression of $Na_v1.6$ is required for β1-mediated neurite outgrowth (30).

β subunits also modulate α subunit function, with effects including alterations in peak I_{Na} density, voltage-dependence of activation and inactivation, rate of inactivation, and persistent and resurgent I_{Na} in cell- and tissue-specific patterns (reviewed in (4)). Early observations indicated that co-expression of β1 with $Na_v1.2$ led to increases in peak I_{Na}, increased rate of inactivation, and hyperpolarizing shifts in activation and inactivation (10). Since that time, extensive study of β subunit function in heterologous systems and mouse models has shown that β subunit effects vary based on the α subunit being modulated as well as the specific cell type (reviewed in (4)). As the biophysical properties of each α subunit differ, at least in vitro, this offers the potential to finely tailor excitability to specific organ, cellular, or subcellular requirements in a dynamic fashion via specific combinations of α and β subunits. Importantly, β subunits also interact with and functionally modulate some VGKCs, particularly the K_v4 channels and $K_v1.1$ (5–7). The association of β subunits, especially β1, with VGSCs and VGKCs provides a means for channel cross-talk during the AP. Taking this multifunctionality into account, it is no surprise that mutations in β subunit genes result in varied and severe channelopathies.

5. β Subunits are CAMs

Channel modulation represents only one facet of β subunit function. The presence of an Ig loop in the structure of all five β subunit proteins predicted a role in cell adhesion and placed β subunits within the IgSF of CAMs, one of the four primary superfamilies of adhesion molecules along with integrins, cadherins, and selectins (17). As CAMs, β subunits engage in both *trans*-homophilic and heterophilic adhesion. Importantly, β subunit-mediated cell adhesion can occur with or without expression of a VGSC α subunit in vitro, although adhesive interactions are critical to the promotion of α subunit cell surface expression (33; 34). β1 engages in extracellular homophilic cell adhesion as well as heterophilic adhesive interactions with VGSC β2, contactin-1, Nf-186, Nf-155, NrCAM, N-cadherin, and the extracellular matrix protein tenascin-R in vitro (33; 35; 43–45). It is predicted that β1B, which shares the N-terminal domain including the Ig loop with β1, engage in similar adhesive interactions as β1. Because β1B is a secreted molecule that can participate in long distance adhesive interactions, it may play roles in novel signaling functions in development, including acting as a soluble antagonist for cell adhesive interactions between cells. The cell adhesive functions of the other β subunits are less extensively characterized. β2 can interact with β1 and tenascin-C, but not contactin, in vitro (34; 45; 46). The ability of β3 to engage in adhesive events is controversial, with studies demonstrating that β3 does (47) and does not (48) homophilically induce cellular aggregation in vitro. Thus, the adhesive capacity of β3 may depend on the expression system and the presence or absence of other CAMs. The role of β4 in cell adhesion remains unclear, although β4 was not observed to mediate neurite outgrowth compared to β1 in vitro (39).

Side Bar

VGSC β subunits were the first non-pore forming ion channel subunits discovered to be CAMs that are able to function both in the presence and absence of the ion-conducting pore. Could this be a trend among other ion channel subunits? A promising candidate for future investigation is calcium channel α2δ. This non-pore-forming VGCC subunit is a

transmembrane protein with an extensive extracellular domain. The presence of a Von Willebrand factor-A domain in α2δ, similar to some integrins, suggests that it may participate in adhesive events (156). Another potential candidate is the Kv4 accessory subunit, DPP6. Similar to VGSC β1, this transmembrane protein plays roles in cell adhesion and motility, in addition to ion channel modulation, with subsequent effects on hippocampal synaptic development and function (157).

β subunit-mediated adhesion plays a role in multiple physiological and developmental processes. Adhesion is critical in axonal fasciculation and axon pathfinding during development. *Scn1b* null mice display defective fasciculation in the corticospinal tract and cerebellar parallel fibers, disorganization of axons in the cerebellar molecular layer, reduced neuron density in the dentate gyrus granule cell layer, increased proliferation of granule cell precursors in the hilus, and defective axonal extension and misorientation of somata and processes of inhibitory neurons in the dentate gyrus and CA1 (37; 49). The mechanism of *trans* β1-β1-mediated neurite outgrowth requires fyn kinase activity and TTX-S I_{Na} can be mimicked by the binding of secreted β1B to β1 on the neuronal cell surface (12; 30; 37; 39).

III. Channelopathies and Pathophysiology of VGSC β Subunits

To summarize to this point, VGSC β subunits are multifunctional, with the capacity to signal through multiple pathways in multiple tissues on multiple timescales. Thus, it is not surprising that a growing number of β subunit gene mutations have been linked to various neuronal and cardiac diseases (Fig. 1).

1. Epilepsy

Epilepsy is characterized by aberrant electrical activity in brain leading to seizures that are recurrent and unprovoked (50). It is estimated that 1% of the U.S. population is afflicted with some form of epilepsy, which can be a consequence of genetic mutation or brain injury (50). Genes encoding VGSCs, including the β subunits, have been implicated in multiple forms of epileptic encephalopathies. The first *SCN1B* mutation identified in epilepsy was p.C121W, which disrupts a key disulfide bond involved in maintaining the Ig fold (51). p.C121W is causative in Generalized Epilepsy with Febrile Seizures plus (GEFS+), in which patients first experience febrile seizures which then progress to persistent afebrile seizures. GEFS+ is one of multiple epilepsies on the pediatric genetic epilepsy spectrum, ranging from mild idiopathic epilepsies to severe forms such as Dravet Syndrome (52).

Dravet Syndrome (DS), formerly known as Severe Myoclonic Epilepsy of Infancy, is a devastating pediatric epileptic encephalopathy (53). Similar to GEFS+, patients typically present with febrile seizures beginning under one year of age that do not resolve, eventually converting to recurrent afebrile seizures of multiple etiologies that are frequently pharmacoresistant. Major comorbidities of DS include early developmental delay and regression, cognitive impairment, high risk of sudden unexpected death in epilepsy (SUDEP), sleep impairment, and ataxia (54). Although the incidence of severe seizures decreases over time, DS leads to a persistent and debilitating phenotype that extends throughout the life of the patient. Many patients continue to experience generalized tonic-

clonic seizures and ataxia. Cognitive impairment does not resolve with age, and there may be a correlation between the severity of cognitive impairment and the severity and frequency of persistent seizures. Many patients are unable to live independently and must rely on support from caregivers or institutionalization (55).

Classically, DS is considered to be a *SCN1A*-linked disease, with over 80% of DS patients carrying heterozygous *de novo* mutations in *SCN1A* that lead to $Na_v1.1$ haploinsufficiency (52). However, a small but growing number of DS patients have mutations in *SCN1B*. The first homozygous *SCN1B* mutation identified in DS was p.R125C, which prevents normal trafficking of β1 to the cell surface, resulting in a functional null phenotype. Interestingly, normal surface localization can be rescued at lowered temperatures in vitro, suggesting possible therapeutic interventions (56). A second homozygous *SCN1B* DS mutation, p.I106F, was subsequently described (57). Heterozygous mutations (p.R85C, p.R85H, p.R125L), as well as a five amino acid deletion (IVS2-2A>C), in *SCN1B* are associated with other epilepsies, including GEFS+ and other febrile seizure disorders (51; 58–60). A mutation specific to β1B, p.G257R, linked to idiopathic epilepsy, was also found to be trafficking-deficient in vitro (12). The majority of *SCN1B* epilepsy mutations identified to date, with the exception of p.G257R, cluster around and within the Ig loop, suggesting that the cell adhesive functions of β1/β1B are clinically relevant (Fig. 1).

Mouse models and in vitro studies have contributed to a clearer understanding of the molecular mechanisms underlying *SCN1B*-linked epilepsy. *Scn1b* null mice have frequent spontaneous generalized seizures, display aberrant neuronal excitability, and have defects in neuronal development (28; 37; 49). Importantly, although seizure onset in these mice begins at approximately postnatal day (P)10, abnormalities in brain development are observed as early as P5 (49). These observations suggest that structural alterations, aberrant cell adhesive interactions, and abnormal excitability early in development may be causative factors in epileptogenesis in *Scn1b* null animals. A knock-in mouse model of p.C121W-mediated GEFS+ displays hyperexcitability in specific subpopulations of central neurons, reduced dendritic arborisation of subicular pyramidal neurons, and increased susceptibility to febrile seizures (31; 61). This is consistent with in vitro studies demonstrating that effects of p.C121W on VGSC gating and kinetics are worsened at higher temperatures, leading to hyperexcitabilty, increased peak current, and increased channel availability (62). Interestingly, p.C121W abrogates normal β1-mediated adhesion without altering the ability of β1 to modulate VGSC function in vitro (63).

Lastly, although *Scn2b* null mice express only 50% of normal cell surface VGSCs in brain and are more susceptible to pharmacologically induced seizures (27). In contrast, patients with cardiac arrhythmia do have *SCN2B* mutations (64; 65), suggesting that altered β2 function may have a larger impact in heart or in cardiac innervation compared to brain.

2. SUDEP and Sudden Death

Epilepsy patients are at increased risk of sudden death compared to the general population. SUDEP is defined as sudden death that is not a result of trauma or drowning, does not result from status epilepticus, and in which post-mortem examination does not uncover a toxicological or anatomical cause (66). SUDEP is estimated to occur in 7.5–17% of all

epilepsy patients (67). The risk of SUDEP increases with the severity of the epilepsy: across all epilepsy patients the annual risk is 0.1%, whereas in DS patients, this risk increases to a mean annual rate of 0.6% (54). Across childhood epilepsies, the risk of sudden death is increased 24-fold compared to the general population (67). These drastic increases in risk of sudden death point to the need for a clearer understanding of the mechanisms that underlie SUDEP as well as effective therapeutic approaches for prevention and intervention.

While some risk factors for SUDEP have been proposed, there are currently no reliable biomarkers. The age and severity of seizure onset correlate with a higher SUDEP risk (68). While a limited number of retrospective studies have linked SUDEP to generalized convulsive seizure episodes, it is likely that death is triggered by seizure-related or associated alterations in multiple organ systems. These include autonomic dysfunction, cardiac arrhythmia, central or obstructive apnea, pulmonary edema, and hypoventilation (69). Consequently, it is critical to establish effective control of seizures, whether through pharmacological or surgical intervention; this is supported by observations that SUDEP risk increases with pharmacoresistance of seizures and non-compliance with anti-epileptic drug therapy (68).

Animal models of DS and other epilepsy disorders, spanning multiple genetic mutations, are also models of SUDEP. The *Scn1b* null mouse is a model of DS and SUDEP, displaying severe spontaneous seizures, ataxia, developmental delay, and premature death by ~P21 (28). *Scn1a*$^{+/-}$ mice model the haploinsufficiency typically seen in human DS patients with de novo *SCN1A* mutations and exhibit a severe seizure phenotype with SUDEP (70). Other channelopathy models of epilepsy display SUDEP, including the VGKC *Kcna1* ($K_v1.1$) null mouse, with severe seizures and sudden death observed in ~25% of pups by P21 (71). The mechanism of SUDEP has been proposed to be "arrhythmia of brain and heart," leading to not only seizures but also cardiac dysfunction, dysregulation of respiratory centers in the brain, and autonomic dysregulation (72). During or following a seizure, patients may experience cardiac events including arrhythmia, tachycardia, bradycardia, T-wave alteration, asystole, or atrial fibrillation (73). The autonomic nervous system is a key regulator of cardiac function and autonomic alterations occur in both focal and generalized seizures and in both ictal and interictal periods (74). Epileptic discharges may propagate to the autonomic nervous system, altering vagal tone and producing downstream discharges onto the heart that can lead to arrhythmias and other potentially fatal cardiac events (69; 73). In animal models, cardiac and autonomic pathologies are frequently observed. Interestingly, these appear to vary between models, such that animals presenting with similar seizure and SUDEP phenotypes display mechanistic differences that may be informative in the elucidation of the etiology of sudden death. *Scn1b* null mice have increased cardiac I_{Na} and prolonged QT and RR intervals on the electrocardiogram (ECG) of anesthetised animals (75). *Kcna1* null mice display atrioventricular (AV) block, bradycardia, premature ventricular contractions and altered HRV (71). *Scn1a*$^{+/-}$ mice have increased cardiac I_{Na}, similar to *Scn1b* null mice, and display bradycardia, focal discharges, R-R variability, and bundle branch block but not AV block on ECGs obtained by telemetry of conscious animals (76). AV block in both *Kcna1* null mice (71) and *Scn1a*$^{+/-}$ (77) mice is removed with atropine, pointing to excessive parasympathetic tone, whereas *Scn1b* null mice do not display changes in QT interval

following treatment with either atropine or propranolol (75), suggesting that the *Scn1b* cardiac phenotype may not be caused by autonomic changes.

Sudden Infant Death Syndrome (SIDS) describes sudden unexpected death in an infant between the ages of one week and one year, where post-mortem analysis does not uncover a clear cause of death (78). The etiology of SIDS, as with SUDEP, remains unclear. Public education efforts in recent years to prompt changes in sleep position in infants have led to a significant reduction in SIDS incidence. However, after this decrease, the number of SIDS cases has remained static, suggesting the influence of non-environmental risk factors (79).

An estimated 10% of SIDS cases are associated with mutations in ion channel genes; within this population, approximately 50% are linked to mutations in *SCN5A* (79). Other ion channel genes mutated in SIDS include *KCNQ1*, *KCNE1*, *KCNH2,* and *RYR2* (80), all of which carry mutations identified in SUDEP (81). This overlap in implicated genes draws a clear parallel to genetic causes of SUDEP as well as cardiac channelopathies. Mutations in VGSC β subunit genes have been linked to SIDS, including *SCN3B* (p.V36M, p.V54G) and *SCN4B* (p.S206L) (82). One of the two identified mutations in *SCN3B*, p.V54G, has also been linked to idiopathic ventricular fibrillation (83). The *SCN4B* mutation p.S206L leads to increased persistent I_{Na} and AP duration (APD) prolongation, a phenotype similar to a separate *SCN4B* mutation (p.L179F) associated with cardiac dysfunction (82; 84). The dual expression patterns of *SCN3B* and *SCN4B* in brain and heart strengthen the proposed involvement of both systems in this disease.

The involvement of various brain regions in SIDS pathophysiology has been extensively investigated, including hippocampus, cerebellum, medulla, and brainstem (reviewed in (85)) and suggest some similarities to *Scn1b*-linked disease models. Thickening of the cerebellar external granule layer (EGL) and abnormal persistence of the granule cell layer into later stages of development has been reported in SIDS, suggesting deficits in granule cell migration in cerebellar development (86), similar to effects seen in *Scn1b* null mouse brain (49). Multiple reports have shown astrogliosis in SIDS brainstem (85). β1 protein is known to be upregulated in reactive astrocytes in human brain in both epileptic and non-epileptic patients (87; 88). Lastly, diffusely delayed myelination in cerebellum and brainstem is observed in SIDS brain (86; 89). *Scn1b* null mice have deficiencies in central nervous system (CNS) myelination in optic nerve, where the thickness of the myelin sheath and formation of normal tight adhesive junctions at the paranodal axo-glial interface are both abnormal (28). While these observations in SIDS patients have not yet been associated with mutations in genes encoding any of the VGSC β subunits, they may inform new lines of investigation, including possible links between SIDS and SUDEP.

SIDS, sudden death, and cardiac arrhythmia may be linked via mutations in the cardiac VGSC complex (90). A connection between SIDS and Brugada Syndrome (BrS) is supported by a mutation in β1B (p.R214Q) present in two adult BrS patients as well as one incident of SIDS (91). This mutation affects two channel types in distinct manners. When co-expressed with $Na_v1.5$, critical in cardiac excitability, β1B p.R214Q results in $Na_v1.5$ loss-of-function. When co-expressed with $K_v4.3$, normally involved in generation of I_{to}, β1B p.R214Q leads instead to gain-of-function (91). This dual modulation is consistent with

previous in vitro and in vivo studies demonstrating functional effects of *Scn1b* on both VGKCs and VGSCs (6; 7; 92). In non-epileptic adults, Sudden Unexpected Death Syndrome (SUDS) and Sudden Unexpected Nocturnal Death Syndrome (SUNDS) are also linked to mutations in *SCN5A* and, less frequently, to *SCN1A* (93). Two mutations in *SCN1B* (p.V138I, p.T189M) and one in *SCN3B* (p.A195T) were identified in a SUNDS patient cohort from China; however, the clinical relevance of these mutations has not yet been established (94).

Taken together, these observations offer the potential for common mechanisms linking various forms of sudden death across all ages from neonate to adult. The presence of VGSC β subunit gene mutations in a subset of these cases suggests these proteins may play key roles in pathophysiology and etiology of sudden death.

3. Cardiac Arrhythmia

In normal cardiac function, VGSCs are responsible for rapid influx of Na^+ during the AP upstroke. In addition, they contribute to conduction velocity throughout the myocardium and participate in excitation-contraction coupling within the myocyte (95; 96) VGSC β subunits are expressed throughout the heart as well as in the conduction system. In human heart, *SCN1B* expression is higher in atria and endocardium than in ventricles and epicardium, whereas *SCN2B* and *SCN3B* expression patterns are found in all regions of the heart (97). At the intercalated disc of ventricular myocytes, the predominant cardiac VGSC, TTX-R channel $Na_v1.5$, has been shown by immunofluorescence to be expressed with β2, β4, and tyrosine-phosphorylated β1 (35; 98–100). At the t-tubules of ventricular myocytes, the TTX-sensitive (TTX-S) channels $Na_v1.1$, $Na_v1.3$ and $Na_v1.6$ are co-expressed with non-phosphorylated β1, β2, and β3 (35; 98; 100; 101). As a result, β subunits are positioned to contribute to all aspects of normal VGSC-linked cardiac function. This suggests that mutant VGSC β subunit proteins and/or alterations in expression levels are likely to contribute to the pathophysiology of cardiac channelopathies.

VGSC β subunit gene mutations are linked to multiple forms of arrhythmia, as recently reviewed (102). The first cardiac-associated mutations identified in VGSC β subunits were *SCN1B* mutations linked to BrS (103), which carries a high risk of sudden cardiac death due to ventricular fibrillation (VF). Multiple BrS-associated mutations have now been identified in *SCN1B* (p.E87Q, β1B p.H162P, p.W179X, p.R214Q) (91; 103; 104), *SCN2B* (p.D211G) (64), and *SCN3B* (p.L10P, V110I) (105; 106). These mutations are linked to reductions in TTX-R I_{Na} density and may include hyperpolarized inactivation kinetics or alterations in rate of recovery from inactivation, exacerbating the severity of the $Na_v1.5$ loss-of-function phenotype. This is similar to *SCN5A*-linked BrS mutations, which result in $Na_v1.5$ loss-of-function (107). One identified *SCN1B* BrS mutation also leads to increased I_{to} by modulation of $K_v4.3$ channels, providing the first human in vivo evidence for a functional link between VGSC β1/β1 subunits and VGKCs (91). Atrial fibrillation (AF) also occurs in patients with β subunit gene mutations, including *SCN1B* (p.R85H, p.D153N, β1B p.R214Q) (65; 108), *SCN2B* (p.R28W, p.R28Q) (65), *SCN3B* (p.R6K, p.L10P, p.M161T, p.A130V) (105; 108; 109), and *SCN4B* (p.V162G, p.I166L) (110). One case of idiopathic ventricular fibrillation has also been identified with a mutation in *SCN3B* (p.V54G) (83). In

general, these mutations lead to decreased $Na_v1.5$-generated I_{Na} and altered channel kinetics. In several of the *SCN3B* mutations, this loss of function is postulated to result from the failure of $Na_v1.5$ cell surface trafficking (82; 83; 105; 106). Interestingly, one *SCN1B* AF-linked mutation has also been described in an epilepsy patient (60; 65), further supporting a phenotypic link between brain and heart disease resulting from VGSC β subunit gene mutations.

Long QT syndrome (LQTS) is characterized by delayed cardiac AP repolarization that can be measured by prolongation of the QT interval on the ECG, leading to increased risk of ventricular fibrillation that can result in sudden cardiac death (111). At least 15 forms of LQTS have been identified, each with specific associated genes, variations in penetrance and allele dominance, and comorbidities. Genes with identified LQTS mutations frequently encode ion channel subunits, including several VGKCs as well as $Na_v1.5$. Two gain-of-function mutations in VGSC β subunit genes have also been identified. The *SCN4B* missense mutation p.L179F has been linked to LQT10, in which a positive shift in I_{Na} inactivation leads to increased window I_{Na} and increased late I_{Na}, consistent with aberrant AP repolarization (84; 112), and similar to the phenotype of the *SCN4B* p.S206L mutation linked to SIDS (82). A recent report also identified a LQTS-associated mutation in β1B, p.P213T (113). Interestingly, although β1B and β4 do not share similar binding mechanisms to VGSC α subunits or have a high degree of sequence homology, the *SCN1B* mutation leads to a similar phenotype to that of *SCN4B*, resulting in altered window I_{Na}, increased channel availability, and increased late I_{Na} (84; 113). Thus, these effects appear to converge despite the structural differences between β1B and β4, perhaps suggesting functional interactions between these two subunits.

The importance of VGSC β subunits in heart has also been demonstrated in null mouse models. *Scn1b* null hearts exhibit increased APD and prolonged QT intervals, consistent with LQTS, that persist after pharmacological autonomic blockade (75). *Scn1b* null ventricular myocytes also display increased transient and persistent peak I_{Na}, with increases in both $Na_v1.5$ expression and ^{3}H-saxitoxin binding, suggesting increased TTX-R and TTX-S VGSC expression (75). *Scn3b* null mice display multiple cardiac abnormalities in both atria and ventricles, including increased susceptibility to arrhythmia and conduction abnormalities that may constitute a BrS model. Loss of β3 in these animals leads to reduced peak I_{Na}, bradycardia, AV block, and alterations in SA node recovery (114; 115). In contrast to *Scn1b* null animals, however, *Scn3b* null nice do not display an overt neurological phenotype, suggesting that the primary functional role of *Scn3b* in vivo may be cardiac.

4. Pain

VGSC α and β subunits are key components of nociceptive and neuropathic pain pathways. Nociceptive pain occurs in response to noxious stimuli in the absence of inflammation or nerve injury, and is mediated by sensory neurons extending from the dorsal root ganglia (DRG), including both small nonmyelinated C fibres and thinly myelinated Aδ fibres (116). DRG neurons express all five VGSC β subunit genes, with mRNA for *Scn1b* being the most abundant (117–119). There is heterogeneity of β expression between the three classes of DRG neurons: small C fibers contain high levels of *SCN3B* mRNA and low levels of

SCN1B, *SCN2B,* and *SCN4B*, while medium and large A fibers express high levels of *SCN1B* and *SCN4B* but low levels of *SCN2B* and *SCN3B* (118) (Table 1). DRG neurons also express both TTX-S ($Na_v1.1$, $Na_v1.2$, $Na_v1.6$, and $Na_v1.7$) and TTX-R ($Na_v1.8$ and $Na_v1.9$) VGSCs, with $Na_v1.7$, $Na_v1.8$, and $Na_v1.9$ as the highest among these (118).

Whereas nociceptive pain permits the appropriate detection of noxious stimuli, neuropathic pain represents a dysregulation of normal sensory and pain pathways, resulting in allodynia. This may be associated with damage or disease, and lead to changes in the structure or molecular composition of pain pathway components, including those in the sensory neurons and spinal cord, to produce persistent pain sensation (116). There are a variety of animal models available for the study of pain, including acute and chronic pain, inflammatory pain and neuropathic pain. Alterations in VGSC subunit expression occur in multiple models, demonstrating the importance of these proteins in the normal regulation of excitability in sensory neurons and tissues receiving input from peripheral nerve.

SCN1B loss-of-function is implicated in pain. *Scn1b* null DRG neurons are hyperexcitable, consistent with observations in central neurons, exhibiting a complex phenotype that includes decreased persistent I_{Na}, a hyperpolarizing shift in inactivation and slowed recovery from inactivation, and decreased surface expression of $Na_v1.9$ (120). Taken together, these observations suggest that global loss of *Scn1b* leads to allodynia. In contrast, in a model of chronic constrictive nerve injury, *Scn1b* mRNA was increased in the laminae of the spinal cord dorsal horn (121). Thus, the phenotypic outputs of genetic deletion of *Scn1b* vs. nerve injury in the presence of *Scn1b* expression appear to be different, perhaps implicating roles for other genes that are up- or down-regulated in the different models.

Evidence for the role of *SCN2B* in pain has provided contrasting results, offering the intriguing possibility for differential VGSC regulation by β2 between specific pain pathways and cell types. *Scn2b* null mice demonstrate reduced sensitivity to inflammatory and neuropathic pain paradigms (117; 122). β2 protein is rapidly increased without concurrent increases in mRNA in both injured and, to a lesser extent, adjacent non-injured neurons in both a spared nerve injury and a spinal nerve ligation model in rat (122). However, *Scn2b* mRNA expression is decreased in cervical sensory ganglia following avulsion injury (123), and decreased in spinal cord dorsal horn in chronic constrictive nerve injury in a rat model (121). Lastly, *Scn2b* deletion reduces TTX-S I_{Na}, particularly $Na_v1.7$, in small DRG neurons as well as modulates kinetics of TTX-S I_{Na} activation and inactivation, without significant effects on TTX-R channels (117).

Upregulation of *Scn3b* mRNA has been observed in multiple pain models. Changes in expression are fiber-type specific and model- dependent. In a chronic constrictive injury model in rat, *Scn3b* mRNA was increased in small C-fibers, in which *Scn3b* is normally highly expressed (26). In contrast, in the streptozotocin model of diabetic neuropathy in rat, *Scn3b* mRNA was increased instead in normally low-expressing medium Aδ fibers and lumbar spinal cord, but not in C fibers (124). *Scn3b* mRNA is also increased in the spared nerve injury model of neuropathic pain in rat as well as in small and medium fibers in the sciatic nerve transection model of axotomy (125).

Little data exist to support a role for *SCN4B* in pain. However, a study of the inherited pain syndrome Paroxysmal Extreme Pain Disorder (PEPD), in which *SCN9A* mutations lead to increased excitability, showed a synergistic effect between disease-associated mutations and β4 in heterologous systems (126). The C-terminal domain of β4 contributes to the generation of resurgent I_{Na} in both cerebellar Purkinje neurons and DRG neurons following peptide cleavage and subsequent generation of a short peptide to induce channel blockade (127). PEPD-linked *SCN9A* mutations result in significant increases in $Na_v1.7$-generated resurgent I_{Na} in the presence of the β4 pore-blocking peptide, implicating β4 as a modulator of neuronal hyperexcitability in this pain syndrome (126; 128).

Overall, the involvement of β subunits in pain pathways position them as potential candidates for therapeutic intervention that may offer more subtle regulation than drugs targeting VGSC α subunits.

5. Demyelinating and Neurodegenerative Disorders

Neurodegenerative disorders involve progressive neuronal loss or damage, and may be associated with other phenomena including pathological protein deposition and lesion development in distinct brain regions, potentially leading to atrophy and functional deficits (129). Loss of myelin or deficiencies in remyelination can confound attempts to restore normal conduction even if the neuron is preserved, leading to aberrant electrical signalling (130). Several lines of evidence implicate VGSC β subunits in demyelinating and neurodegenerative disorders. *Scn1b* null mice display defects in central myelination, with decreased numbers of optic nerve nodes of Ranvier, increased axonal degeneration, spinal cord dysmyelination, and disruption of nodal ultrastructure in both CNS and peripheral nervous system (PNS). At nodes of Ranvier, loss of β1-mediated adhesion appears to be sufficient to disrupt proper formation of paranodal junctions; eversion of the last paranodal loop adjacent to the nodal gap is observed, resulting from loss of the tight septate-like junctions formed by adhesive interactions involving β1, contactin-1, Caspr, and Nf-155 that maintain proper axo-glial contact at the paranodes (28). In complementary evidence for the importance of these nodal protein-protein interactions, *Cntn1* null mice display decreased β1 protein expression (37). Taken together, these data suggest that maintenance and/or establishment of myelination are mediated in part by β1 cell adhesive interactions. Axonal electrical activity is also a key factor in normal myelination (131); it is not yet known, however, if the channel modulation function of β1 contributes to these mechanisms.

In contrast to *Scn1b*, *Scn2b* null mice appear to have normal myelination (27; 29). However, *Scn2b* deletion is neuroprotective in a mouse model of Multiple Sclerosis (MS), with decreased axonal degeneration, fewer demyelinated and dysmyelinated axons, increased survival, and reduced symptom severity (29). In MS and other neurodegenerative disorders, aberrant VGSC α subunit expression is observed, including redistribution of channels from normal cellular subcompartments and altered levels of channel expression. These changes are proposed to contribute to neurodegeneration via abnormal influx of Na^+ into damaged axons, particularly via increased $Na_v1.6$ expression, which displays higher levels of persistent I_{Na} compared to other neuronal VGSCs. This is then postulated to lead to reverse activation of the sodium-calcium exchanger (NCX) and increased local intracellular calcium

concentrations that activate downstream injury cascades, axonal degeneration, and neuronal loss (130). Interestingly, cerebrospinal fluid (CSF) from MS patients exhibits decreased BACE1 activity, suggesting decreased β2 cleavage and consequent alterations in normal VGSC α expression and localization. Low BACE1 activity in MS is linked to worsened disease severity and longer disease duration, and BACE1 expression continues to decrease throughout disease progression (132).

Altered VGSC β subunit expression and protein modification have been observed in studies of human neurodegenerative disease, including amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), and Huntington's disease (HD). ALS is characterized by degeneration of motoneurons in the spinal cord, motor cortex, and brainstem, and ALS neurons are hyperexcitable, potentially implicating VGSCs in disease pathology. At P120 in the *Sod1* mouse model of ALS, an age consistent with the adult onset typical of human ALS, the level of *Scn8a* mRNA is decreased in spinal cord, concurrent with decreased *Scn1b* mRNA and increased *Scn3b* mRNA and protein in ventral dorsal horn. These changes provide a potential molecular basis for hyperexcitability (133). In a PD mouse model, *Scn4b* mRNA and protein were increased, including higher expression of a more heavily glycosylated form of β4 that displayed developmental regulation during disease progression (134). Lastly, *SCN4B* mRNA and protein are downregulated in the striatum of HD patients and mice before the onset of motor symptoms, a hallmark brain region in HD that typically exhibits progressive neuronal loss. Decreases in β2 protein levels also occur in this mouse model, but significantly later than changes in β4 (135). The importance of β4 in HD pathology may be a consequence of neuritic degeneration secondary to β4 downregulation, as overexpression of β4 in vitro leads to neurite outgrowth and branching, dendrite thickening and increased numbers of dendritic spines (135).

VGSC β subunits are targets for sequential proteolytic cleavage by BACE1 and γ-secretase, offering potential links to the pathophysiology of Alzheimer's disease (AD) and neurodegeneration in addition to normal development. BACE1 is ubiquitously expressed in brain, and its expression increases with age as well as in AD patient cortex. In AD pathology, BACE1 and γ-secretase cleave APP to release the Aβ peptide, which then accumulates to form aggregates in brain (136). Interestingly, BACE1 cleavage of β2 reverses normal β2 effects on VGSC α subunit modulation, and in BACE1 null mice, decreased cleavage of β2 in brain may contribute to increased neuronal excitability (137). AD patients are at increased risk of seizure (138), which may be partly predicated on VGSC-mediated alterations in brain excitability following cell surface downregulation, and may affect both excitatory and inhibitory neurons (41). Finally, *SCN3B* mRNA is decreased in AD brains that contain deleterious neurofibrillary tangles (NFT), a key pathological feature of AD alongside Aβ-containing plaques, as compared to both non-NFT patient brain and control brain tissue (139).

6. Cancer

VGSCs are implicated in multiple types of cancer, particularly in the development of metastatic potential, or the ability of the tumour cell to decrease adhesive interactions with neighbouring cells and become motile (140). The identity of the predominant VGSC α

subunit expressed varies between cancer types; e.g., $Na_v1.5$ has been implicated in breast and ovarian cancer, whereas the critical channel subtype in prostate cancer is $Na_v1.7$ (141) and $Na_v1.6$ in cervical cancer (142). Increased VGSC α expression correlates with increased metastasis, and pharmacological blockade of I_{Na} with TTX abrogates tumour cell invasive capability in vitro (140)

Consistent with its known functions in cell adhesion, *SCN1B* has been implicated in cancer, particularly breast cancer, and may play an important role in metastasis. In breast cancer, expression of β1 is high in weakly metastatic and low in highly metastatic cell lines in vitro (143). In contrast, in prostate cancer, *SCN1B* mRNA is increased in highly metastatic compared to weakly metastatic epithelial cell lines (141). In an implanted tumour model in mouse, β1-expressing tumours displayed increased growth rate and size with corresponding decreases in survival rates. Cells from these tumours showed decreased levels of apoptosis, enhanced angiogenesis, and metastasis to both lung and liver (36). In cervical cancer biopsy tissue, *SCN1B* mRNA expression is decreased (142), whereas in human breast, *SCN1B* mRNA is increased in cancerous compared to non-cancerous tissue (36). Reminiscent of *SCN1B* function in neurite outgrowth, alterations in the morphology of β1-expressing breast cancer cells are observed in vitro, including process extension that requires expression of the β1 Ig domain as well as activation of fyn kinase (36). Thus, similar to brain, in which the functional roles of *SCN1B* appear to vary with neuronal cell type (140), the roles of *SCN1B* in cancer may be tissue specific.

Of the four β subunit genes, *SCN1B* expression predominates in many cancers. Other β subunits may also be important, although these observations have not yet been assessed for pathogenicity or clinical relevance. A high-throughput screen of post-metastasis samples from late-stage colorectal cancer patients identified two separate heterozygous missense mutations in *SCN3B*, p.Q89L and p.A195T (144). *SCN3B* is also upregulated in human cancer cell lines after DNA damage (145). In prostate cancer cell lines, mRNA for all four β subunit genes is present, although, again, expression of *SCN1B* predominates. In highly metastatic cell lines in vitro, *SCN4B* mRNA expression is decreased (141). In cervical cancer biopsy tissue, *SCN3B* mRNA levels are increased while both *SCN2B* and *SCN4B* are decreased. Interestingly, for *SCN2B* and *SCN4B*, differing results were observed between primary culture and biopsy samples, indicating the importance of evaluating expression in native contexts (142).

Taken together, these data suggest VGSC β subunit genes may be biomarkers in cancer as well as future therapeutic targets for the treatment of metastatic cancer. The differences in β subunit expression among cancer types, however, point to the importance of understanding the molecular mechanisms of β subunit function in order to tailor therapies appropriately.

7. Autism Spectrum and Mood Disorders

Autism spectrum disorders (ASDs) are comorbidities of DS (54) and some cases of GEFS + (146). Thus, a promising avenue of investigation is ion channel expression in ASDs and other neuropsychiatric disease including mood disorders and psychiatric disorders, as many of these diseases share common genetic foundations. ASDs are phenotypically diverse, but also have ~90% heritability, indicating the significance of genetic components (147). There

is a high degree of overlap between ASD and epilepsy: an estimated 15–35% of pediatric epilepsy patients are autistic, and conversely, 7–46% of autistic patients display some form of epilepsy (148). VGSCs, VGKCs, and voltage-gated calcium channels (VGCCs) have all been associated with ASD and mood disorders, as well as other genes important in neural plasticity and synaptic function (147). *Scn1a*$^{+/-}$ mice, which exhibit impaired GABAergic transmission and behavioral defects similar to those seen in ASD, including stereotypy and impaired social interaction, had ameliorations in these behaviors after treatment with the $GABA_A$-R modulator clonazepam (149). No cases of VGSC β subunit mutations have yet been reported with direct association to ASD; however, given both the importance of β subunits on VGSC function as well as the emergence of *SCN1B* mutations in DS, it is likely that such a linkage may be found in future patient cohorts.

β1-interacting proteins are also associated with neuropsychiatric disorders. Multiple mutations have been found in *ANK3*, encoding ankyrin-G, which interacts with the C-termini of β1 and β2 in multiple cellular subcompartments including the AIS and nodes of Ranvier in neurons (150). A gain-of-function mutation has been identified in *KCND2*, encoding the β1-interacting VGKC $K_v4.2$, in autistic twins who also exhibited seizures (148). Lastly, genetic variation including copy number variation and deletion has been reported in three members of the contactin gene family of CAMs, *Cntn4*, *Cntn5*, and *Cntn6* (151). ASD-linked mutations have not yet been reported in *Cntn1*, a known heterophilic binding partner of β1; however, *Cntn5* null mice show decreased β1 protein expression in brain and, conversely, *Scn1b* null mice show increased Cntn5 protein expression, suggesting reciprocal modulation (H. O'Malley, unpublished observations).

IV. Concluding Remarks

To conclude, VGSC β subunits are more than auxiliary. At the molecular level, these multifunctional proteins play roles in VGSC and VGKC modulation, *trans* and *cis* adhesion, and modulation of gene expression. At the tissue level, they are critical in neuronal development, including neuronal proliferation, migration, and pathfinding, neurite extension, axonal fasciculation, and myelination, and thus the regulation of excitability. In heart, β subunits are proposed to play similar roles in cell-cell communication at the intercalated disc, where multi-protein complexes of VGSCs and VGKCs co-exist, as well as modulation of channel complexes linked to calcium handling at the t-tubules. Mutations in the genes encoding β subunits are linked to a myriad of channelopathies, including epilepsy, sudden death syndromes like SUDEP and SIDS, and cardiac arrhythmia. Furthermore, changes in β subunit expression may modulate pain, demyelinating and neurodegenerative disorders, cancer, and autism spectrum and mood disorders. Clearly, the VGSC β subunits are an untapped reservoir of novel therapeutic potential.

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Acronyms and Definitions

AD	Alzheimer's disease
AIS	axon initial segment
ALS	amyotrophic lateral sclerosis
ASD	autism spectrum disease
BACE	beta-site APP cleaving enzyme
BrS	Brugada Syndrome
DRG	dorsal root ganglion
DS	Dravet Syndrome
GEFS+	Generalized Epilepsy with Febrile Seizures Plus
HD	Huntington's disease
IgSF	Immunoglobulin superfamily
MS	Multiple Sclerosis
NCX	sodium-calcium exchanger
PD	Parkinson's disease
PEPD	Paroxysmal Extreme Pain Disorder
SIDS	Sudden Infant Death Syndrome
SUDEP	Sudden Unexpected Death in Epilepsy
SUDS	Sudden Unexpected Death Syndrome
SUNDS	Sudden Unexpected Nocturnal Death Syndrome
TTX	tetrodotoxin

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Summary Points

- Sodium channel β subunits are multifunctional molecules that signal through multiple signaling pathways on multiple time scales, with and without the pore-forming α subunits.
- Sodium channel β subunits are Ig superfamily cell adhesion molecules.
- Sodium channel β subunits play roles in sodium channel modulation, sodium channel trafficking and localization, and cellular migration, proliferation, and process outgrowth.
- Sodium channel β subunits associate with and modulate potassium channels.
- Sodium channel β subunits are substrates for sequential cleavage by β- and γ-secretases and, through this mechanism, modulate gene expression.
- Mutations in the genes encoding β subunits are linked to a myriad of channelopathies, including epilepsy, sudden death syndromes like SUDEP and SIDS, and cardiac arrhythmia. Furthermore, changes in β subunit expression may modulate pain, demyelinating and neurodegenerative disorders, cancer, and autism spectrum and mood disorders.
- VGSC β subunits are an untapped reservoir of novel therapeutic potential.

Future Issues

1. Are all VGSCs heterotrimers? Which α and β subunits associate in specific tissues in vivo?
2. Does the α/β subunit composition of VGSCs change in pathology?
3. How is the splicing of *SCN1B* regulated?
4. How is the sequential cleavage of β subunits by BACE and γ-secretase regulated?
5. Are β subunits expressed in the absence of α subunits in vivo and if so, in which cell types?
6. What is the most effective way to target β subunits for the development of novel therapeutics?

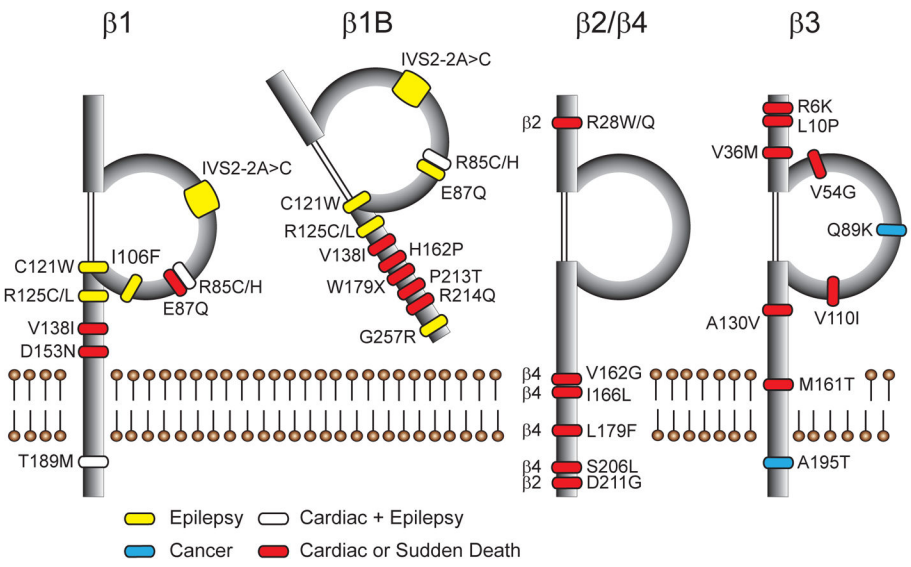

Figure 1.

Disease-linked mutations in VGSC β subunits. Sites of mutated amino acids for each of the five β subunit protein products are denoted, including epilepsy (yellow), cardiac disease and non-SUDEP sudden death (red), cancer (blue), and mutations which have been identified in patients with multiple diagnoses (white). In β1/β1B, epilepsy-linked mutations cluster around the Ig loop, while no mutations have so far been identified in the Ig loop of β2 or β4, but instead occur within the transmembrane domain and C-terminus. Mutations in β3 occur throughout the protein.

Table 1

Expression profiles of VGSC β subunits in brain, peripheral nerve, and heart. Adapted from (4).

	β1	β1B	β2	β3	β4
Nervous system					
Hippocampal neurons	(28)		(16; 27)	(15; 26)	(16; 21)
Cortical neurons	(5)	(11; 13)	(16; 27)	(15; 26; 152)	(16; 21)
Purkinje neurons	(30)	(11; 13)	(16; 27)	(26)	(16; 21)
Cerebellar granule neurons	(30; 39)				
Astrocytes	(87) (reactive) (153; 154)(cultured)		(153; 154)(cultured)	(153)(cultured)	
Bergmann glia	(39)				(39)
Nodes of Ranvier	(28; 43)		(27)		(21)
Dorsal root ganglia	(117–119)	(11; 13)	(16; 117; 118; 122)	(15; 26; 118; 152)	(16; 21; 118)
Heart					
Ventricular myocytes	(100)		(100)	(100)	(100)
Atrial myocytes	(97; 98)		(98)	(98)	(98)
Sino-atrial node	(155)		(155)	(155)	(155)